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Cytochrome P450-Catalyzed Binding of 3-Methylsulfonyl-DDE and o,p′-DDD in Human Adrenal *Zona Fasciculata/Reticularis*

ÖRJAN LINDHE, BRITT SKOGSEID, AND INGVAR BRANDT

Department of Environmental Toxicology (Ö.L., I.B.), Uppsala University, Norbyvägen 18A, S-752 36 Uppsala, Sweden; and Endocrine Oncology Unit (B.S.), Department of Medical Sciences, University Hospital, S-751 85 Uppsala, Sweden

3-Methylsulfonyl-2,2′-bis(4-chlorophenyl)-1,1′-dichloroethene ($MeSO_2$-DDE) is a potent, tissue-specific toxicant that induces necrosis of the adrenal *zona fasciculata* following a local CYP11B1-catalyzed activation to a reactive intermediate in mice. Autoradiography was used to examine CYP11B1-catalyzed binding of $MeSO_2$-[^{14}C]DDE and the adrenocorticolytic drug 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichlorethane; (o,p′-[^{14}C]DDD, Mitotane, Lysodren) in human adrenal tissue slice culture. Both compounds gave rise to a selective binding in the one sample of normal adrenal *zona fasciculata/reticularis*, leaving *zona glomerulosa* and the adrenal medulla devoid of binding. Addition of the CYP11B1 selective inhibitor metyrapone (50 μM) reduced $MeSO_2$-[^{14}C]DDE binding below the detection limit, whereas o,p′-[^{14}C]DDD binding was reduced only by 42%. Selective binding of $MeSO_2$-[^{14}C]DDE and o,p′-[^{14}C]DDD was also observed in an aldosterone-producing adrenocortical carcinoma and in a nonfunctional adrenocortical hyperplasia. Exposure of slices from the normal adrenal cortex to $MeSO_2$-DDE (25 μM) resulted in an increased accumulation of 11-deoxycorticosterone, 11-deoxycortisol and androstenedione in the medium, and exposure to o,p′-DDD (25 μM) did not alter the steroid secretion pattern. No histological changes were found in either $MeSO_2$-DDE- or o,p′-DDD-exposed slices, compared with nonexposed slices. We suggest that $MeSO_2$-DDE might act as a potent adrenocorticolytic agent in humans. Further studies are needed to establish the usefulness of $MeSO_2$-DDE as a possible alternative for the treatment of adrenocortical hypersecretion and tumor growth. (*J Clin Endocrinol Metab* 87: 1319–1326, 2002)

THE ADRENOCORTICOLYTIC ACTIVITY of 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichlorethane; (o,p′-DDD, Mitotane, Lysodren) was first described in 1949 in dogs (1). o,p′-DDD has subsequently proved to be a tissue-selective toxicant, following local metabolic activation and irreversible protein binding in the adrenal cortex in several species including human (2), dog (3), domestic fowl (4), and mink (5). By virtue of its tissue-selective toxicity, o,p′-DDD is currently used as an adrenocorticolytic drug for treatment of adrenocortical carcinoma and Cushing's syndrome (6–8). The effective dose for treatment of adrenocortical cancer gives a plasma concentration higher than 14 μg/ml (44 μM), whereas plasma concentration below 10 μg/ml (31 μM) seems therapeutically insufficient (9, 10). Only one-third of the patients (194 of 551) who were not cured by surgery, responded to o,p′-DDD treatment (7). In addition to hypocortisolism, o,p′-DDD gives rise to dose-dependent side effects in the gastrointestinal tract (nausea, vomiting, and diarrhea) and central nervous system (dizziness and headache). Treatment-related unspecific effects such as weakness and fatigue are also observed (10–12). In a substantial proportion of patients, these side effects are intolerable at therapeutic doses, and the drug has had to be withdrawn.

Aryl methyl sulfones of DDE and PCBs were first identified in blubber of Baltic gray seal (13). The sulfones form in the mercapturic acid pathway, involving sequential metabolic transformation during enterohepatic circulation (14). Several of the methyl sulfones are characterized by a highly cell- and tissue-selective distribution pattern in the body. 3-Methylsulfonyl-2,2′-bis(4-chloro-[^{14}C]phenyl)-1,1′-dichloroethene ($MeSO_2$-[^{14}C]DDE) was originally found to give rise to a cell-specific irreversible binding in mouse adrenal *zona fasciculata* cells *in vivo* (15). $MeSO_2$-DDE was subsequently demonstrated to be a highly potent adrenal toxicant that induces mitochondrial degeneration and cell death following a CYP11B1-catalyzed metabolic activation in the murine adrenal cortex (15–17). Adrenocortical cell death and reduced plasma corticosterone levels is seen in fetal and suckling mice following exposure of the pregnant or lactating dam to $MeSO_2$-DDE (18–20). The toxicity of $MeSO_2$-DDE in the fetal adrenal *zona fasciculata* is effectively blocked by maternal metyrapone treatment in mice (20). A relationship among time, dose, and extent of *zona fasciculata* damage is evident in adult mice (17). As demonstrated by metabolic binding studies using the mitochondrial fraction of human adrenal homogenate from four subjects, the human adrenal cortex also seems capable of forming irreversibly bound $MeSO_2$-[^{14}C]DDE-protein adducts (21).

The dechlorinated DDT metabolite DDE is the major persistent environmental pollutant present in human blood and breast milk worldwide (22). In the past decade, high DDE levels have been reported in Mexico (23, 24), Brazil (25), Ukraine (26), and Zimbabwe (27). In Swedish breast milk samples, the level of p,p′-DDE is correlated to that of $MeSO_2$-DDE (360 and 1.5 ng/g lipid, respectively) (28, 29). The

Abbreviations: BG, Background; CYP, cytochrome P450; DMSO, dimethylsulfoxide; $MeSO_2$-DDE, 3-methylsulfonyl-2,2′-bis(4-chlorophenyl)-1,1′-dichloroethene; o,p′-DDD, 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichlorethane; PSL, photo-stimulated luminescence.

corresponding levels of p,p′-DDE and $MeSO_2$-DDE in Mexican breast milk are as high as 13,900 and 130 ng/g lipid, corresponding to about 15 nmol $MeSO_2$-DDE/liter milk (Bergman, Å., personal communication).

We have recently described a simple precision-cut tissue slice culture procedure, with which to examine $MeSO_2$-DDE-induced irreversible binding as well as functional and morphological changes in the adrenal cortex (30). In the present study, we used this procedure to examine cytochrome P450-catalyzed irreversible binding of $MeSO_2$-[^{14}C]DDE and o,p′-[^{14}C]DDD in human adrenal tissue *ex vivo*.

Materials and Methods

Adrenal tissue

Normal adrenal tissue, cortex as well as apparently unaffected medulla, was obtained from a 34-yr-old female MEN type 2 patient operated on for an ipsilateral pheochromocytoma.

Tissue from a lymph node metastasis of an aldosterone-producing adrenocortical carcinoma was obtained from a 54-yr-old male.

Tissue from a bilateral nonfunctioning adrenocortical hyperplasia was obtained from a 58-yr-old female.

Informed consent was given by all three patients.

Chemicals

$MeSO_2$-[^{14}C]DDE (495 MBq/mmol), unlabeled $MeSO_2$-DDE, and o,p′-[^{14}C]DDD (414 MBq/mmol) were kindly donated by Åke Bergman (Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden) (31, 32). As determined by gas chromatography mass spectroscopy, radiochemical purity values were more than 99%. Tetracosactide (Synacten depot, 1 mg/ml) was obtained from Ciba (V. Frölunda, Sweden), dimethylsulfoxide (DMSO), and agarose (type VII, low melting temperature) were from Sigma (St. Louis, MO). Methacrylate Technovit 7100 was obtained from Kulzer (Wehrheim, Germany). All liquids and dyes were from Merck and Co. (Darmstadt, Germany) except chloroform, which came from Prolabo (Paris, France). Liquid film NTB 2 was purchased from Kodak (Rochester, NY).

Preparation and incubation of tissue slices

Adrenal tissue was placed in ice-cold isotonic saline solution after removal and kept on ice until embedded in 3% agarose. Precision-cut slices (200 μm) were prepared in a Krumdieck tissue slicer (Alabama Research and Development, Munford, AL) in ice-cold PBS (33). Slices of about equal size were placed in six-well plates containing culture medium on a titanium screen holder and incubated for 24 h (after a 1-h preincubation; 1 slice/well; four to five wells per treatment), as described elsewhere (30).

To inhibit CYP-dependent enzyme activity in the slices, incubation wells were supplemented with the CYP11B1 (11β-hydroxylase) inhibitor metyrapone (50 μM) when applicable. To stimulate ACTH-regulated enzyme activity in the slices, the synthetic ACTH-analog tetracosactide (11 nM) was added. The labeled and unlabeled test substances ($MeSO_2$-DDE and o,p′-DDD) were added to the fresh incubation medium, dissolved in DMSO (not exceeding 0.5% of the total volume). Control slices were cultured in medium containing DMSO only.

Autoradiography

Microautoradiography. Slices were incubated with $MeSO_2$-[^{14}C]DDE (2.6 μM, 1.3 kBq/ml) or o,p′-[^{14}C]DDD (3.8 μM, 2.2 kBq/ml) for 24 h. Following incubation, slices were fixed overnight in buffered formaldehyde (4%). The fixed slices were dehydrated, embedded in methacrylate, sectioned, and dipped in liquid NTB2 film emulsion, as described elsewhere (30). Autoradiograms were exposed (4 C) for 1 yr to enable localization of irreversible binding in metyrapone-treated slices. Autoradiograms were developed, stained, and examined as previously described (30).

Radioluminography. Semiquantification of tissue-bound radioactivity was accomplished by apposing tissue sections to imaging plates (BAS-Ip MP 2040S, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 49 d. The radioactivity in the labeled areas of the adrenal sections was recorded by reading the imaging plate in a Phosphoimager (BAS 1500, Fuji Photo Film Co., Ltd.) (34, 35). For semiquantification of the tissue-bound radioactivity, a Windows-based bioimaging analyzer program (Image-Gauge, version 3.122, Fuji Photo Film Co., Ltd., Tokyo, Japan) was used.

To correlate tissue-bound radioactivity and metabolically active regions in the incubated slices, the labeled areas of the images were marked selectively at 1 pixel resolution (1 pixel = 100 μm). Values obtained were expressed as photo-stimulated luminescence (PSL) minus background (BG) per square millimeter of 2-μm-thick tissue sections (PSL-BG)/mm^2). The values were adjusted according to the difference in specific activity of the two compounds.

To check the imaging plates with respect to exposure linearity, the same plate was repeatedly exposed to the same sections for 7, 14, 28, 49 and 122 d, reading and erasing the plate between each exposure. To examine interexposure variation, the imaging plate was exposed repeatedly (three times) for 7 d, reading and erasing the plate between each exposure. Intraexposure variation was measured on three adjacent sections on the same glass slide.

Hormone analysis

Cortisol, 11-deoxycortisol, corticosterone, 11-deoxycorticosterone, aldosterone, androstenedione, and 17-OH-progesterone concentrations in the medium after 24 h of culture were measured with HPLC using UV detection (241 nm), as described previously (30). The steroid products were separated using a linear gradient of 18–80% acetonitrile (1 ml/min), obtained by diluting acetonitrile/tetrahydrofuran (90/10 vol/vol) with methanol/water (40/60 vol/vol) for 32 min. The amounts of steroids were expressed as nanomole per slice. The detection level of cortisol/corticosterone was 5 pmol/ml medium. To adjust for differences in slice size, the steroids were expressed as a percentage of the amount of cortisol from the same slice.

Histology

$MeSO_2$-DDE and o,p′-DDD (dissolved in DMSO) were added to the wells in amounts corresponding to a final concentration of 25 μM in the medium. Following incubation, slices were embedded in methacrylate and prepared for light microscopy as above. For reference purposes, some adrenal slices were fixed directly after sectioning.

Statistical evaluation of data

Statistical analysis performed using one-way ANOVA (Bonferroni's multiple comparison test as the posttest) to analyze hormone concentrations, *t* test to analyze bound radioactivity, and linear regression test to analyze exposure linearity. Significance was assigned a value of P less than 0.05. All tests were performed with Prism software version 3.0 for Windows (GraphPad Software, Inc., San Diego, CA).

Results

Autoradiography

As determined by light microscopy, autoradiograms of adrenal slices exposed to $MeSO_2$-[^{14}C]DDE (Figs. 1 and 2, A and B) or to o,p′-[^{14}C]DDD (Fig. 3, A and B) showed a high and selective labeling of *zona fasciculata* and *zona reticularis* in the one sample of normal adrenal cortex. The labeling of *zona reticularis* was markedly stronger than that of *zona fasciculata*. *Zona glomerulosa* and the adrenal medulla were devoid of bound radioactivity exceeding that of the background level (Figs. 2, E and F, and 3, E and F).

As determined by radioluminography, no significant difference in the amount of tissue-bound radioactivity between $MeSO_2$-[^{14}C]DDE- and o,p′-[^{14}C]DDD-exposed slices could be detected (Fig. 4). The images of tissue-bound radioactivity

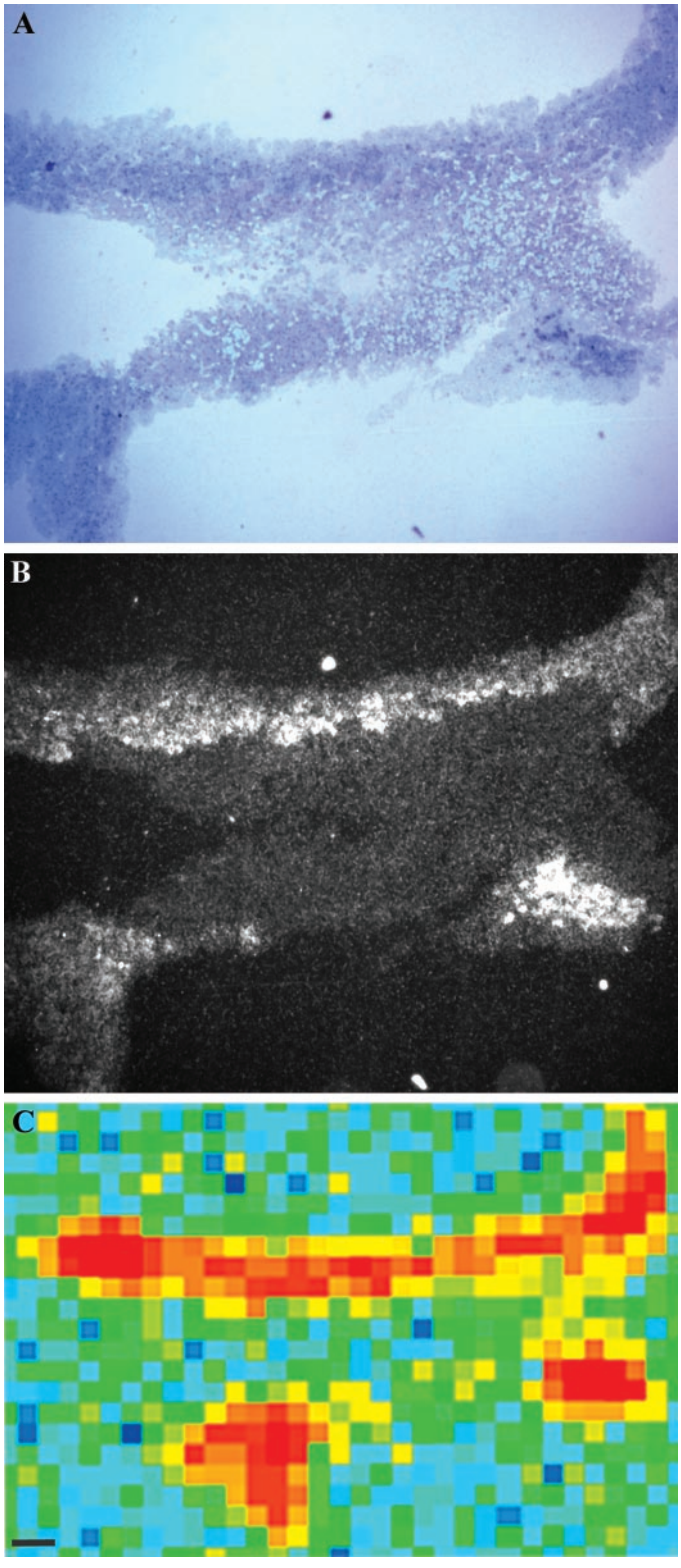

FIG. 1. Overview of $MeSO_2$-[^{14}C]DDE binding in the human adrenal cortex *ex vivo*. An image of the tissue-bound radioactivity semiquantified with radioluminography (C) closely matches the image of the corresponding microautoradiogram (B). A, Original histological section stained with toluidine blue (50-fold magnification, bar = 200 μm).

semiquantified with radioluminography closely matched the images of the microautoradiograms (Fig. 1). In slices exposed to metyrapone and $MeSO_2$-[^{14}C]DDE, binding in *zona fasciculata/reticularis* was inhibited below the detection limit ($P < 0.05$) at 49 d of exposure (Figs. 2, C and D, and 4). In metyrapone-exposed slices, irreversible o,p′-[^{14}C]DDD-binding in *zona fasciculata/reticularis* was inhibited by 42 plus or minus 12% (± SEM; $P < 0.05$), compared with slices exposed only to o,p′-[^{14}C]DDD (Figs. 3, C and D, and 4).

With increasing exposure time, the slopes of the linear regression lines for $MeSO_2$-[^{14}C]DDE and o,p′-[^{14}C]DDD differed significantly from that of the background ($r^2 > 0.96$; $P < 0.01$). Variation in (PSL-BG)/mm^2 values between different exposures of the same $MeSO_2$-[^{14}C]DDE-exposed slice was less than 8% (35.6 ± 2.8; mean ± SEM). Intraexposure variation was less than 2% (113.8 ± 1.5; mean ± SEM) for three adjacent sections.

Slices from a lymph node metastasis of an aldosterone-producing adrenocortical carcinoma, exposed to $MeSO_2$-[^{14}C]DDE (Fig. 2, G and H) or to o,p′-[^{14}C]DDD (Fig. 3, G and H) showed a selective binding of both compounds to the adrenocortical carcinoma cells. No labeling above the background level could be observed in surrounding tissues.

In slices from a bilateral nonfunctioning adrenocortical hyperplasia, the levels (for both compounds) of bound radioactivity were about one-third of that in normal adrenal tissue ($P < 0.001$, data not shown). The binding of both compounds was inhibited to the same extent with metyrapone treatment as in the normal tissue.

Steroid hormone secretion

Cortisol, 11-deoxycortisol, corticosterone, 11-deoxycorticosterone, aldosterone, androstenedione, and 17-OH-progesterone were all detected in the culture medium after 24 h. Cortisol and corticosterone were the major secreted steroids, representing 53% and 29%, of the total steroid secretion from nonexposed control slices. In $MeSO_2$-DDE- or o,p′-DDD-exposed (25 μM) slices, no significant difference in cortisol or corticosterone secretion could be observed, compared with control slices. No difference could be observed between $MeSO_2$-DDE- or o,p′-DDD-exposed slices (Fig. 5A). A significant increase in 11-deoxycorticosterone secretion to the medium was observed in $MeSO_2$-DDE-exposed slices, compared with o,p′-DDD-exposed slices and control slices (Fig. 5B; $P < 0.05$). Androstenedione secretion was also increased in $MeSO_2$-DDE-exposed slices, compared with o,p′-DDD-exposed slices (Fig. 5B; $P < 0.05$). 11-Deoxycortisol was detectable only in the culture medium of $MeSO_2$-DDE-exposed slices (Fig. 5B).

Histology

After 24 h of culture, no obvious histological differences could be observed between control slices and $MeSO_2$-DDE- or o,p′-DDD-exposed (25 μM) slices. Compared with slices fixed immediately after slicing, cultured control slices were not visibly different (data not shown).

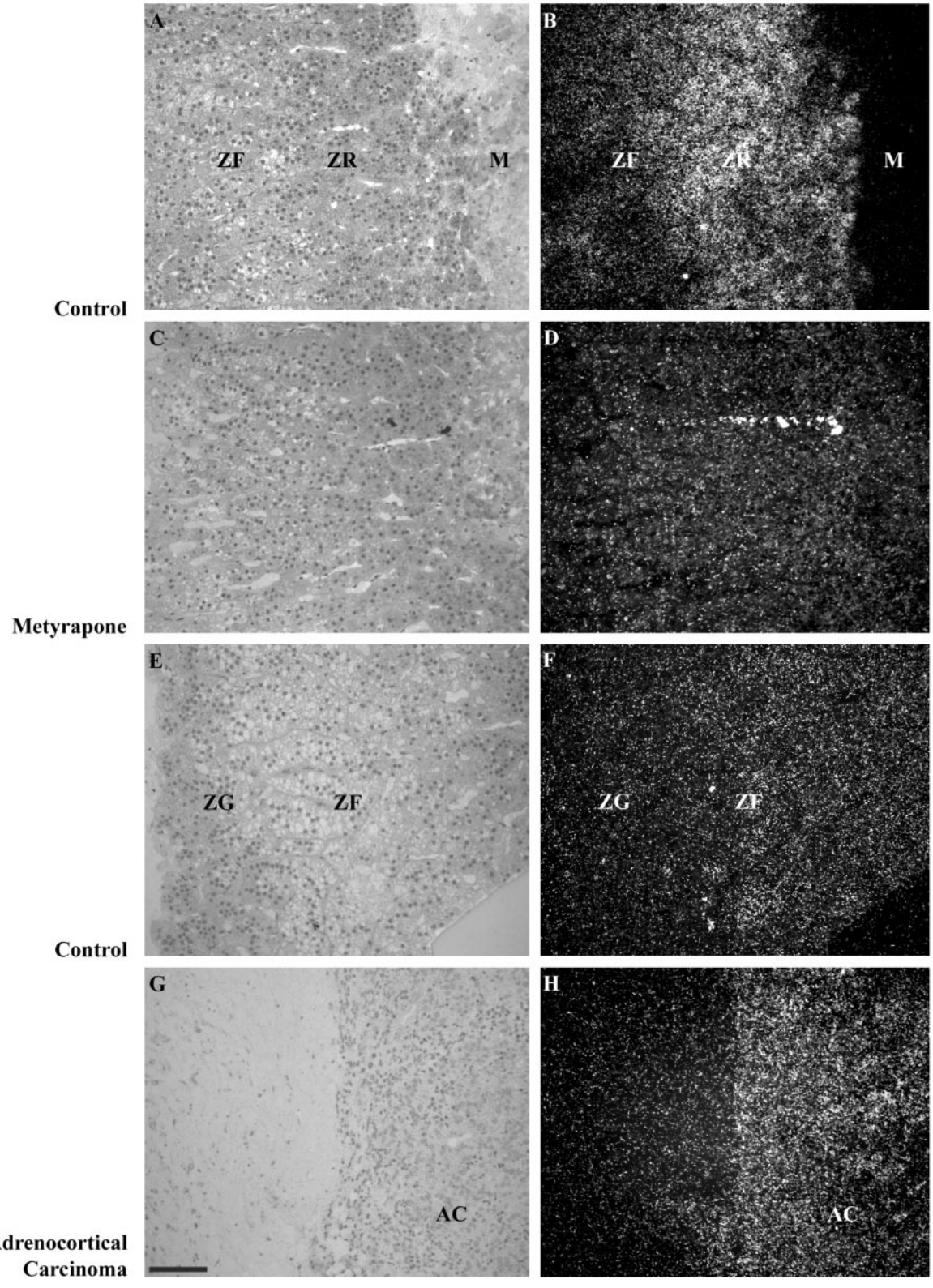

FIG. 2. Irreversible binding of $MeSO_2$-[^{14}C]DDE in *zona fasciculata* and *zona reticularis* in cultured slices from human adrenal cortex (A, C, E, and G, light-field photographs; B, D, F, and H, dark-field photographs). B, A strong labeling of *zona reticularis* and a marked labeling of *zona fasciculata* is observed. Exposure together with metyrapone inhibited $MeSO_2$-[^{14}C]DDE binding in *zona fasciculata/reticularis* almost completely (C and D). No binding of $MeSO_2$-[^{14}C]DDE is observed in *zona glomerulosa* (E and F). Slices from a lymph node metastasis of an aldosterone-producing adrenocortical carcinoma (G and H), exposed to $MeSO_2$-[^{14}C]DDE show selective labeling of adrenocortical cells (AC). In surrounding tissue, no labeling above the background level is visible. ZG, *Zona glomerulosa*; ZF, *zona fasciculata*; ZR, *zona reticularis*; M, adrenal medulla (200-fold magnification, bar = 100 μm).

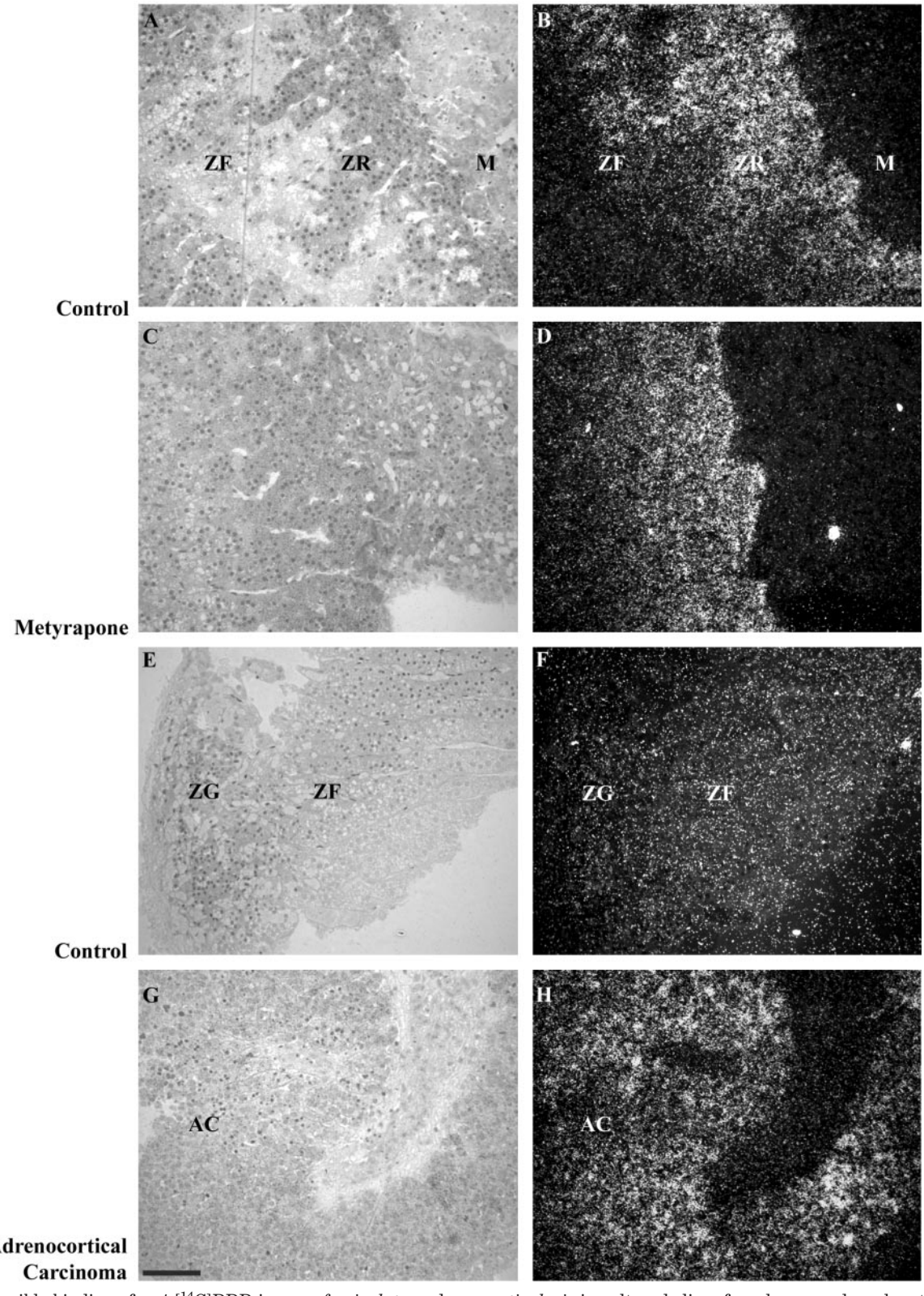

FIG. 3. Irreversible binding of o,p′-[^{14}C]DDD in *zona fasciculata* and *zona reticularis* in cultured slices from human adrenal cortex (A, C, E, and G, light-field photographs; B, D, F, and H, dark-field photographs). B, A strong labeling of *zona reticularis* and a marked labeling of *zona fasciculata* is observed. Exposure together with metyrapone only partly reduced o,p′-[^{14}C]DDD binding in *zona fasciculata*/*reticularis* (C and D). No binding of o,p′-[^{14}C]DDD is observed in *zona glomerulosa* (E and F). Slices from a lymph node metastasis of an aldosterone-producing adrenocortical carcinoma (G and H), exposed to o,p′-[^{14}C]DDD, show selective labeling of adrenocortical cells (AC). In surrounding tissues, no labeling above the background level is visible. ZG, *Zona glomerulosa*; ZF, *zona fasciculata*; ZR, *zona reticularis*; M, adrenal medulla (200-fold magnification, bar = 100 μm).

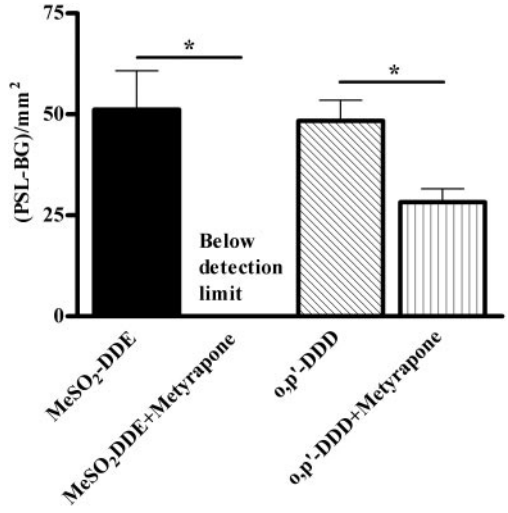

FIG. 4. Quantification of irreversible binding using radioluminography reveals no difference in level of binding of $MeSO_2$-[^{14}C]DDE and o,p′-[^{14}C]DDD in *zona fasciculata/reticularis*. Exposure together with metyrapone inhibited $MeSO_2$-[^{14}C]DDE binding below the detection limit ($P < 0.05$) and reduced o,p′-[^{14}C]DDD binding by 42 plus or minus 12% ($P < 0.05$). (n = 4; mean ± SEM; 49 d of imaging plate exposure.)

Discussion

Our previously reported findings in mouse adrenal tissue exposed to $MeSO_2$-[^{14}C]DDE *ex vivo* showed that strong irreversible binding was confined to *zona fasciculata*. In contrast, o,p′-[^{14}C]DDD-binding was very weak, compared with that of $MeSO_2$-[^{14}C]DDE. Interestingly, however, there was a selective localization of o,p′-[^{14}C]DDD binding to both *zona fasciculata* and *zona reticularis* in the mouse adrenal cortex (30).

In this study we found a strong $MeSO_2$-[^{14}C]DDE-derived binding in human adrenal tissue that was confined to both *zona fasciculata* and *zona reticularis* in a normal cortex. Irreversible o,p′-[^{14}C]DDD-binding was localized in a similar way. Even though $MeSO_2$-[^{14}C]DDE concentration in the medium was almost half that of o,p′-[^{14}C]DDD, the levels of binding were of roughly equal strength. In mouse, the $MeSO_2$-DDE-activating enzyme CYP11B1 is expressed only in *zona fasciculata* (36). In the human adrenal cortex, CYP11B1 is expressed in both *zona fasciculata* and *zona reticularis* but not in *zona glomerulosa*, the adrenal medulla, or the capsule (37, 38). CYP11B1 was also found in an aldosterone-producing adenoma as well as in two incidentally detected adrenocortical adenomas (38). These discrepancies support the contention that CYP11B1 catalyzes activation of $MeSO_2$-DDE to a reactive metabolite also in normal and cancerous human adrenal cortex.

Metyrapone is a potent CYP11B1 inhibitor that blocks synthesis of cortisol from 11-deoxycortisol (86%, 5 μM) in V79 hamster cells transfected with the human *CYP11B1* gene (37). We have recently reported that metyrapone inhibits irreversible $MeSO_2$-[^{14}C]DDE binding and corticosterone secretion in mouse adrenal slice culture and mouse adrenal homogenate (15, 30, 39). Exposure of cultured human adrenal slices to metyrapone (50 μM) reduced irreversible $MeSO_2$-[^{14}C]DDE binding below the detection limit, whereas o,p′-[^{14}C]DDD binding was reduced by only 42%. Provided that

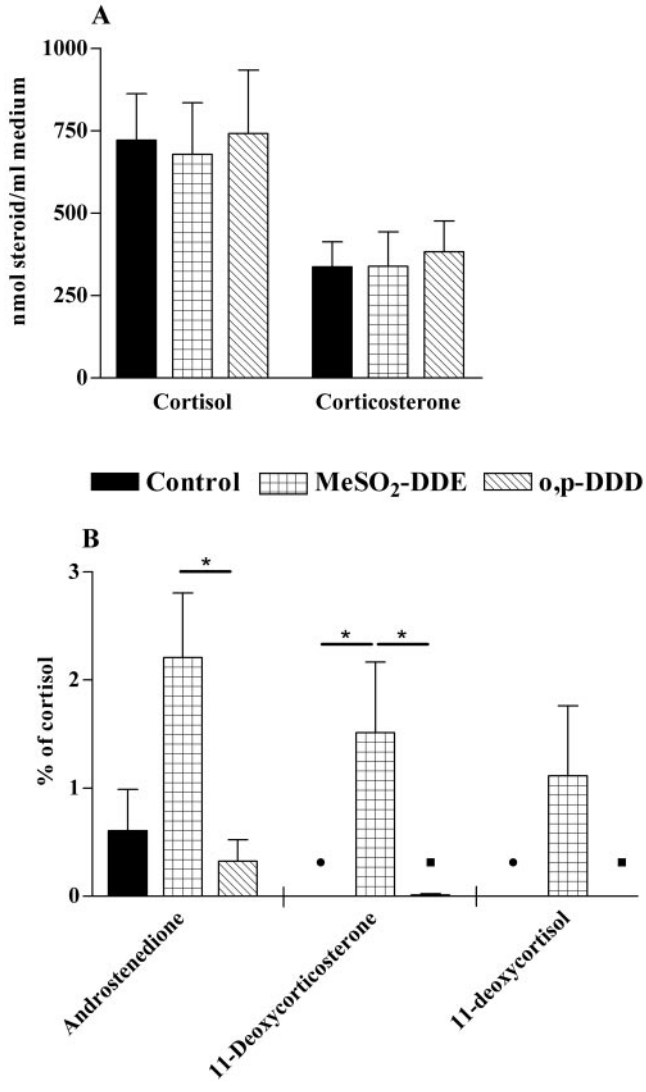

FIG. 5. Levels of steroid hormones in medium after 24 h of culture; in $MeSO_2$-DDE- or o,p′-DDD-exposed (25 μM) slice cultures, no significant differences in cortisol or corticosterone secretion are observed, compared with control slices. No differences between $MeSO_2$-DDE- and o,p′-DDD-exposed slices are observed (A). A significant increase in 11-deoxycorticosterone secretion to the medium is detected in $MeSO_2$-DDE-exposed slices, compared with o,p′-DDD-exposed slices and control slices (B; $P < 0.05$). Androstenedione secretion is also increased in $MeSO_2$-DDE-exposed slices, compared with o,p′-DDD-exposed slices (B; $P < 0.05$). 11-Deoxycortisol is detectable only in the culture medium of the $MeSO_2$-DDE-exposed slices (B) (n = 4; mean ± SEM). ●, Control not detectable; ■, o,p′-DDD not detectable or barely detectable.

the metabolic activation of o,p′-DDD is mainly dependent on CYP11B1, a more complete inhibition of binding would be expected. The observed inhibition of o,p′-[^{14}C]DDD binding therefore supports the contention that another enzyme was also involved in the activation of o,p′-DDD in both human and mouse adrenal cortex. In mouse, CYP11B2 is expressed only in *zona glomerulosa* (36). Because no binding was observed in *zona glomerulosa,* either in human or mouse adrenal tissue, CYP11B2 was probably not involved in the metabolic activation of o,p′-DDD. If another enzyme in the steroid synthesis chain is involved, it seems likely that o,p′-DDD

binding would occur also in other steroid-secreting organs, such as testis and ovary. Indeed, we have preliminary findings showing that o,p′-[^{14}C]DDD is bound in rat ovary granulosa cells (Lindhe Ö, unpublished data). More studies regarding the identities of o,p′-DDD-activating enzymes are needed.

Radioluminography proved to be a quick and sensitive way to quantify levels of metabolite binding in the human adrenal cortex. Combined with the exact localization of binding obtained by microautoradiography, radioluminography is an efficient means to semiquantify the levels of bound $MeSO_2$-[^{14}C]DDE and o,p′-[^{14}C]DDD adducts in the target cells (30). The linear increase in PSL/mm^2 values during long exposure times and low exposure-to-exposure variation favors the quantitative use of radioluminography.

The adrenal cortex is complex with regard to the hormone secretion pattern. Adrenal endocrine disrupting substances have several enzymes to target, and the possible effects on homeostasis are numerous. By using precision-cut adrenal slice culture, we show that a number of steroids can be quantified in the culture medium and that changes in secretion pattern can be observed. Slice culture facilitates investigations of effects caused by several compounds in one single individual. This method gave us the opportunity for comparisons of the drug effects *per se* without disturbance of interindividual variation. In our study on slices from one normal adrenal cortex, the concentration of o,p′-DDD (25 μM) did not produce any visible effect on steroid secretion, compared with control. Interestingly, at the same concentration and below the therapeutically effective o,p′-DDD plasma concentration, $MeSO_2$-DDE (25 μM) gave rise to an increased accumulation of 11-deoxycorticosterone, 11-deoxycortisol, and androstenedione in the culture medium. This finding indicates a reduced CYP11B1 activity. In the human adrenocortical carcinoma cell-line H295R, $MeSO_2$-DDE exposure reduced CYP11B1-catalyzed cortisol formation after 24 h (Johansson, M., submitted for publication). Decreased corticosterone plasma levels have also been observed in suckling mouse pups following a single injection of $MeSO_2$-DDE (6 mg/kg) to the lactating dam (18). In homogenate incubations of cells from four specimens of human adrenal cortex, the apparent K_m value of $MeSO_2$-DDE binding to protein was 17 times lower than that of o,p′-DDD (1.4 and 24 μM, respectively) (21). This implies that $MeSO_2$-DDE might be toxic at lower doses than o,p′-DDD. These findings support the contention that $MeSO_2$-DDE is a tissue-selective toxicant in the human adrenal cortex.

We conclude that one can examine steroid secretion from human adrenal slices *ex vivo* and suggest that the slice culture procedure could be useful for evaluating the endocrine disrupting potential of chemicals and pharmaceutical products. We also suggest that $MeSO_2$-DDE be evaluated as a suspected human adrenal toxicant, especially with regard to the risk posed to suckling infants in developing countries. The numbers of human adrenals so far examined are limited and further studies needed. However, considering the low potency and the potentially severe side effects frequently observed following o,p′-DDD treatment (10–12), we propose that $MeSO_2$-DDE should be evaluated as a possible alternative in the therapy of adrenocortical hypersecretion and tumor growth. (A patent application has been filed.)

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Address all correspondence and requests for reprints to: Dr. Ingvar Brandt, Department of Environmental Toxicology, Uppsala University, Norbyvägen 18 A, S-752 36 Uppsala, Sweden. E-mail ingvar.brandt@ebc.uu.se.

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