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Irreversible binding of *o,p′*-DDD in interrenal cells of Atlantic cod (*Gadus morhua*)

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Abstract

Precision-cut tissue slices of the anterior kidney from Atlantic cod (*Gadus morhua*) were prepared with a Krumdieck tissue slicer and exposed to 2-(2-chlorophenyl)-2-(4-chloro-(^{14}C)phenyl)-1,1-dichlorethane (*o,p′*-[^{14}C]DDD) in vitro. Microautoradiography revealed irreversible *o,p′*-DDD-derived binding confined to the glucocorticoid producing interrenal cells (adrenocortical analogues). This cell-selective binding was confirmed by means of autoradiography at different levels of resolution on Atlantic cod administered *o,p′*-[^{14}C]DDD intragastrically. The results provide evidence for a site-specific metabolic activation and irreversible binding of *o,p′*-DDD in the interrenal cells, which, in turn, may modify glucocorticoid homeostasis.

Keywords: *o,p′*-DDD; Atlantic cod; Interrenal cells; Glucocorticoid homeostasis; Adrenocortical analogues

o,p′-DDD is a lipophilic metabolite of *o,p′*-DDT which may modify glucocorticoid homeostasis by giving rise to reactive intermediates capable of macromolecular binding in adrenocortical cells (Hart et al., 1971; Baker and Van Dyke, 1984). However, the ability of adrenocortical cells to metabolise *o,p′*-DDD into toxic intermediates vary between species (Brandt et al., 1992). Accordingly, in situ bioactivation and irreversible binding of *o,p′*-DDD in adrenocortical cells have been observed in human, seal, otter, mink, dog, and chicken adrenal tissue. On the other hand, activation and binding is far less pronounced in the laboratory model species, the mouse and the rat (Brandt et al., 1992; Lindhe et al., 2001). Such species differences in susceptibility, complicates risk assessment and highlights the need for improved laboratory screening tools for the detection of species-specific chemical-induced effects.

Previous studies show that *o,p′*-DDD may impair cortisol secretion also in teleost fish (Ilan and Yaron, 1983; Leblond and Hontela, 1999; Benguira and Hontela, 2000). Whether this effect is mediated through in situ bioactivation of *o,p′*-DDD in the glucocorticoid-secreting interrenal cells (the equivalents of the mammalian adrenal cortex) is not known.

Precision-cut tissue-slice culture provides a test system where cell-specific effects can be studied in vitro, using the species of concern (Krumdieck et al., 1980; Bach et al., 1996). This methodology has been applied to evaluate vitellogenin induction in trout liver slices (Schmieder et al., 2000). Furthermore, we have recently

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reported the use of adrenal slice culture to study irreversible metabolite binding and adrenocorticolytic effects in human and rodent tissue (Lindhe et al., 2001, 2002). We found it pertinent to apply this in vitro procedure in order to provide evidence for a possible interaction of *o,p′*-DDD in the interrenal cells of Atlantic cod.

2-(2-chlorophenyl)-2-(4-chloro-(^{14}C)phenyl)-1,1-dichlorethane (*o,p′*-[^{14}C]DDD; 11.2 mCi/mmol) was kindly prepared by Dr. Åke Bergman, Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden (Bergman and Wachtmeister, 1977; Lindholm et al., 1987). The radiochemical purity was >99%. Tetracosactide (1 mg/ml, Synacten Depot®) was obtained from Ciba (V. Frölunda, Sweden), fluorescamin, dimethylsulfoxide and agarose (Type VII, low melting temperature) from Sigma (St. Louis, MO, USA). Methacrylate Technovit 7100 was obtained from Kulzer (Wehrheim, Germany). All liquids and dyes were from Merck (Darmstadt, Germany). Liquid film NTB2 was purchased from Kodak (Rochester, USA). X-ray films (Structurix D7R) were purchased from Agfa-Gevaert, Antwerp, Belgium.

Atlantic cod (210–245 g) caught outside Fiskebäckskil, Sweden were transported to Uppsala in oxygenated seawater. The fish were kept in 1000 l holding tanks in recirculating water (pH 7.1–7.2; 2.8% salinity; 8–12 °C) at day/night cycles automatically adjusted to the diurnal variations at latitude 51°N for one month before the experiment. They were given a commercial maintenance diet for salmonids (EST 41, Aller, Denmark). Three fish were killed with a blow to the head. The anterior kidneys (containing the interrenal cells) were immediately excised and kept in ice-cold PBS buffer until embedded in agarose (3%). Precision-cut slices (300 μm) were prepared in a Krumdieck tissue slicer (Alabama Research and Development, Munford, Alabama, USA) (Krumdieck et al., 1980) in ice-cold PBS and further processed as described recently (Lindhe et al., 2001). Cultured slices were exposed to *o,p′*-[^{14}C]DDD (6.3 μM, 0.1 μCi/ml) for 24 h. The tissue slices were then fixed in formaldehyde, dehydrated and embedded in methacrylate (Technovit 7100, Heraeus Kulzer GmbH, Wehrheim, Germany). The mounted slices were sectioned (2 μm) and slides carrying the sectioned tissue were dipped in NTB2 liquid emulsion. Following exposure (4 °C) for one year, the autoradiograms were developed and stained with toluidine blue.

For tape section autoradiography of whole fish and for microautoradiography of anterior kidney from fish exposed in vivo six Atlantic cod (110–160 g) were given the test compound by gastric intubation, dissolved in cod liver oil (0.5 ml, 3.6 μmol/kg body weight, 40 μCi/kg).

Three weeks after administration, three fish were killed in a solution of benzocaine in water and frozen in liquid nitrogen. They were then embedded in an aqueous gel of carboxymethylcellulose (1%) and subjected to tape section autoradiography according to Ullberg (1977). All sections were freeze-dried at −20 °C for 48 h. Autoradiograms obtained from film exposed to the freeze-

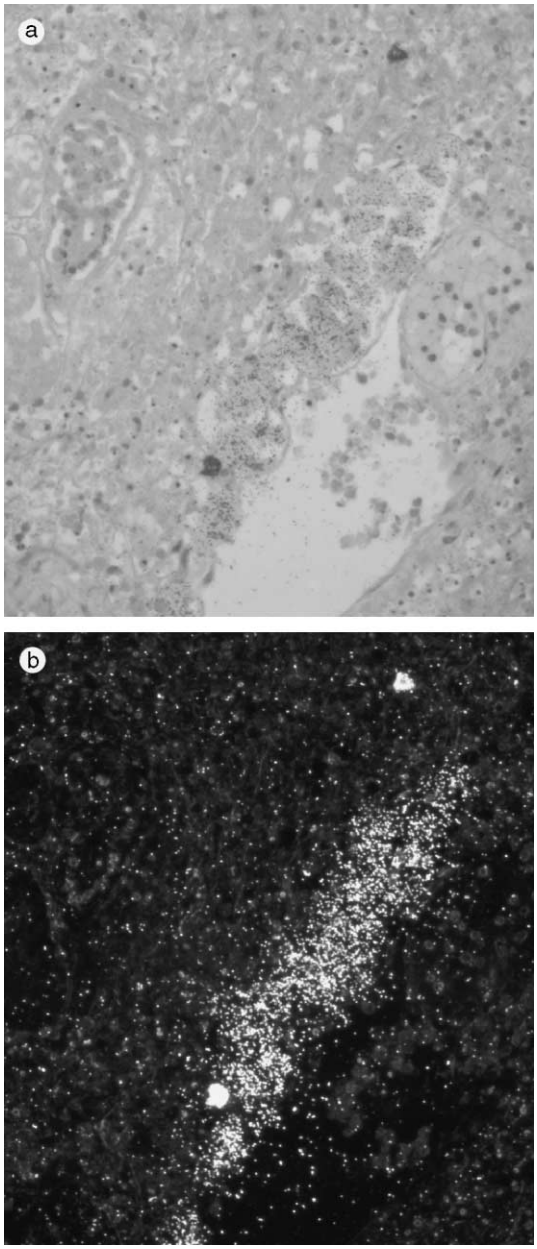

Fig. 1. (a) Histological section and (b) corresponding autoradiogram (dark field photograph) of a precision-cut tissue-slice from the anterior kidney of Atlantic cod (*Gadus morhua*). The slice was exposed to *o,p′*-[^{14}C]DDD for 24 h. Selective binding of radioactivity in the interrenal cells adjacent to a blood vessel is observed (200×).

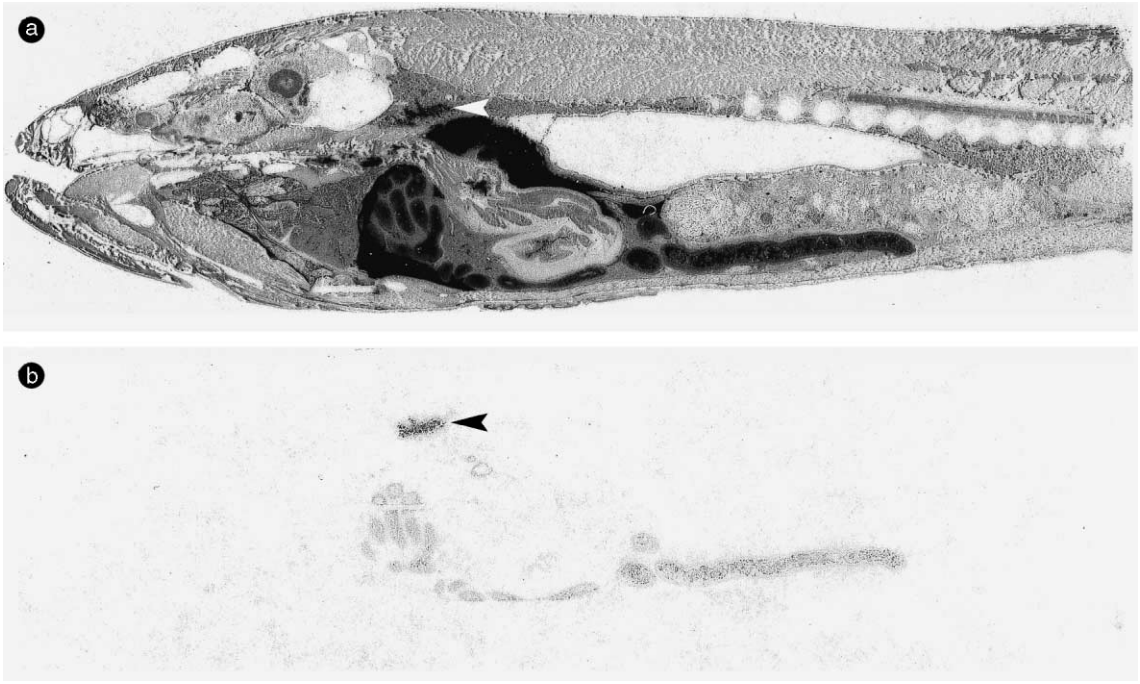

Fig. 2. (a) Tape-section autoradiogram of an Atlantic cod (*Gadus morhua*) killed three weeks after oral administration of *o*,*p*′-[^{14}C]DDD, and (b) autoradiogram of an adjacent section extracted with organic solvents. Note the high level of non-extractable radioactivity in the anterior kidney (arrowheads) (4/5 of natural size).

dried sections represent both unchanged test compound and its metabolites. Furthermore, every second tissue section was extracted consecutively in a series of organic solvents, as previously described (Ingebrigtsen et al., 2000). After washing for 5 min in running water, the sections were dried. Autoradiograms obtained from solvent-extracted sections were considered to represent metabolites of the test compound firmly bound to tissue macromolecules (Brandt and Brittebo, 1989). All sections were apposed to X-ray films (Structurix D7R). The films were developed after three months exposure.

Three weeks after administration three fish, all treated like those for tape section autoradiography, were killed by a blow to the head. The head kidneys were excised and fixed in buffered formaldehyde (4%). They were further processed for microautoradiography in the same way as the tissue slices exposed in vitro. The exposure time of the film emulsion was nine months.

Microautoradiography of precision-cut tissue slices from the anterior kidney exposed to *o*,*p*′-[^{14}C]DDD in vitro revealed that radioactivity was selectively bound in the interrenal cells (Fig. 1(a) and (b)). Furthermore, autoradiography at different levels of resolution performed on cod exposed to *o*,*p*′-[^{14}C]DDD in vivo confirmed this selective localization of non-extractable radioactivity (Figs. 2(a, b) and 3(a, b)). This indicates that the slice culture procedure is relevant for the route of exposure prevailing in the environment. Evidently, the observed selective binding is the result of *o*,*p*′-[^{14}C]DDD being metabolised to reactive intermediates in situ, as was previously demonstrated in human, mink and bird adrenal tissue (Jönsson et al., 1993a,b; Lindhe et al., 2002). Previous reports of *o*,*p*′-[^{14}C]DDD-derived irreversible binding in mammalian adrenocortical cells provide evidence for a partial bioactivation by the enzyme CYP11B1, implying involvement of another enzyme present in the adrenal cortex (Lund, 1994; Lindhe et al., 2001, 2002). The strong degree of homology of the CYP11B-subfamily among vertebrates together with the previous reports of *o*,*p*′-DDD-induced impaired cortisol secretion in teleost fish favour the interpretation that adrenocorticolytic activity is mediated by the same mechanism as in mammals.

In conclusion, a site-specific irreversible binding of *o*,*p*′-[^{14}C]DDD takes place in the glucocorticoid producing interrenal cells in Atlantic cod; this binding may impair homeostasis by modification of cortisol secretion. We propose microautoradiography of cultured precision-cut tissue slices as a useful laboratory-screening tool for evaluation of potential wildlife effects.

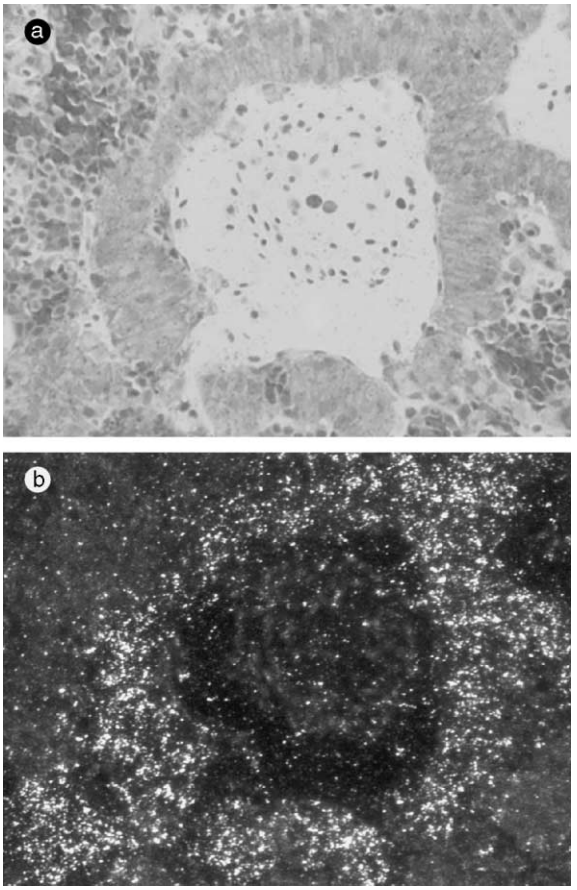

Fig. 3. (a) Histological section and (b) corresponding autoradiogram (dark field photograph) from the anterior kidney of Atlantic cod (*Gadus morhua*) killed three weeks after oral administration of *o*,*p*′-[^{14}C]DDD. Note selective binding of *o*,*p*′-[^{14}C]DDD-derived radioactivity in the interrenal cells surrounding a blood vessel (400×).

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