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FAILURE OF 2 HYDROXYESTRADIOL TO INTERACT WITH DOPAMINE INHIBITION OF HUMAN PROLACTIN SECRETION IN VITRO AND WITH DOPAMINE RECEPTORS OF PROLACTIN-SECRETING ADENOMAS

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SUMMARY. The ability of 2-Hydroxyestradiol, a catecholestrogen, and 17 β Estradiol to interact with the dopamine inhibition of prolactin and with dopamine receptors has been tested on dispersed human prolactin-secreting cells obtained from ten pituitary adenomas. There is a 80 % inhibition of prolactin secretion obtained by addition of dopamine in a superfusion system. This inhibition is not affected by preexposure to the steroids, or by their introduction into the perifusion medium.Moreover 2 Hydroxy-estradiol and 17 β Estradiol do not interact with the binding of ^{3}H Domperidone to DA receptors.

Different studies have recently shown that 2-hydroxyestradiol ($2\text{-}OHE_2$) a naturally occuring metabolite of 17 β estradiol ($17\ \beta\ E_2$) may have an effect on prolactin (PRL) secretion in animals and in humans (1,2). Because of its pharmacological interaction with catecholamines, it has been suggested that this effect should be of physiological interest.

Till now some in vivo studies have demonstrated that this catecholestrogen may act as a dopamine antagonist as plasma PRL was increased after administration of $2\text{-}OHE_2$ in ovariectomized rats (1) and in hypogonadal women (2). By contrast in a recent in vitro study using superfused rat pituitary gland, it has been shown that $2\text{-}OHE_2$ inhibited PRL release and may act as a dopamine agonist (3).

In addition it has been reported that $2\text{-}OHE_2$ was able to interact with dopamine receptors in rat pituitary gland (4).

The present study was undertaken in order to evaluate the in vitro effect of $2\text{-}OHE_2$ on PRL secretion from superfused human PRL-pituitary adenomatous cells.

The interaction between $2\text{-}OHE_2$ and dopamine receptors was also evaluated on the membranes of PRL-secreting pituitary adenomas.

MATERIAL AND METHOD

Ten human PRL-secreting pituitary adenomas were collected in the operating room after selective transsphenoidal adenomectomy (5). Before

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surgery, the diagnosis was established on clinical, radiological and biological criteria. Tissue fragments from four adenomas were immediately placed in an ice cold medium (see below for its composition) to which was added 5.10^{-6}M dopamine to avoid the release of PRL. Fragments from six other adenomas were frozen in liquid nitrogen until use for receptor studies.

^{3}H-Domperidone (SA 23.6 Ci/mmol) was purchased from C.E.A. (Saclay, France). Unlabeled domperidone was a gift of Le Brun Laboratory (Paris, France). Dopamine (DA), 2-Hydroxyestradiol (2-OHE_2), 17 β Estradiol (17 β E_2) and pargylline were purchased from Sigma (St Louis, MO). Bio-Gel P2 (200-400 mesh) was purchased from Bio-rad laboratories (Richmond-Calif.). All other chemicals were purchased from either Merck (Darmstadt, Germany) or Sigma.

Morphological studies

Small tissue fragments were studied by light microscopy and immunochemistry as described previously (6). All the adenomas studied were proved to be PRL-secreting adenomas by light microscopy and immunocytochemistry.

Receptors studies

The interaction between DA receptors, 2OHE_2 and 17 β E_2 was studied using a technique described previously (7). The membranes of the PRL-secreting adenomatous cells were incubated with 3 or 30 nM of ^{3}H-domperidone and various concentrations of 2-OHE_2 and 17 β E_2 (from 10^{-10} to 10^{-4} M).

Dynamic studies in perfused columns

The method used was that described by Yeo et al (8). The adenomas were placed at 0°C in a Krebs Ringer buffer (NaCl 118 mM, KCl 4.7 mM, KH_2PO_4 1.18 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.18 mM, Glucose 11.1 mM) supplemented with 0.5 % bovine serum albumine (BSA). To avoid degradation of DA, 0.1 % ascorbic acid and 10 µM pargylline were added to the buffer. The complete buffer was called perifusion medium. 5.10^{-6} M DA was added to the perifusion medium till the recovery of the cell suspension.

The adenomatous cells were dispersed mechanically using cataract knives and pasteur pipettes. No enzyme was added to the perifusion medium. The cell suspension (4 ml) was then layered on 4 ml of perifusion medium containing 4 % BSA and centrifuged at 70 g for 10 min at 0°C. The pellet was resuspended in 4 ml of perifusion medium without DA and the cells counted after checking of their viability with an 0.1 % trypan blue solution. The cell suspension was mixed with Bio-Gel P2 (0.5 g per 1 ml of cell suspension containing 1 or 2.10^{6} cells). The mixture was then placed at 37° C in a syringe and fractions were collected each 5 minutes (2.5 ml). For one hour, the cells were perfused with the medium alone to allow the cells to reach their basal secretion. The mean value of the PRL content of the six last fractions represented the 100 % of control (C, figs 1,2 and 3). The different drugs were then added to the perifusion medium which was continuously gazed with CO_2/O_2 (5 %/95 %). 2 OHE_2 solution was prepared just before use to avoid its degradation. The fractions were immediately frozen at -20°C after collection and then assayed by RIA for their PRL content as described previously (9).

RESULTS

Receptors studies. Neither 2-OHE_2 nor 17 β E_2 interacted with the binding of ^{3}H domperidone to DA receptors, at any concentration of ^{3}H ligand and drugs tested (Fig. 1).

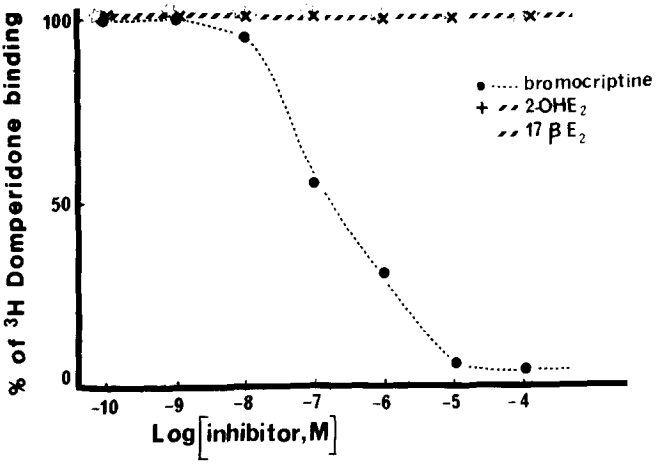

Fig. 1 - Displacement of ^{3}H-Domperidone binding to human PRL-secreting pituitaries by Bromocriptine, 2-OHE$_2$ and 17 βE$_2$. Membrane suspension were incubated for 30 min at 30°C with 30 nM ^{3}H-Domperidone and increasing concentration of inhibition.

Dynamic studies. The results are presented on figs 2 - 4. 2-OHE$_2$ and 17 β E$_2$ had no effect on PRL secretion from dispersed human adenomatous cells. Furthermore when the same cells were later on

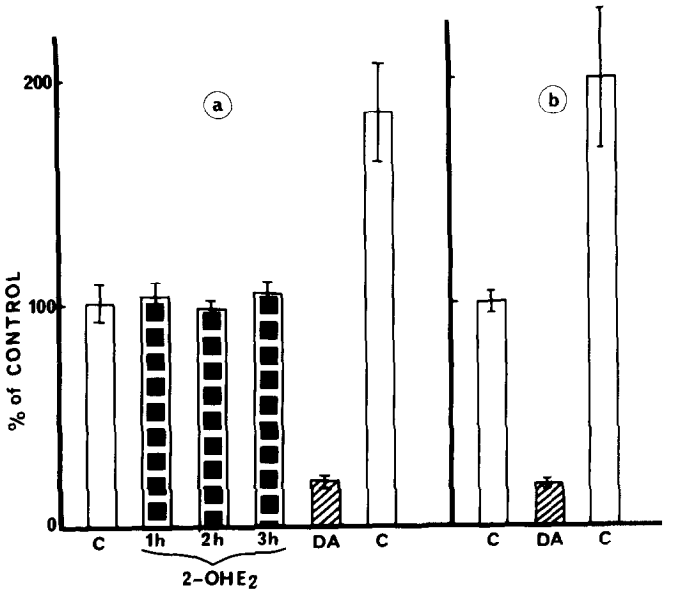

Fig. 2 - In one syringe, PRL-adenomatous cells were exposed to 2-OHE$_2$ (10^{-6}) for 3h. followed by 30 min. with DA (10^{-6}M) and 30 min with control (C) medium (fig. 1a). In another syringe running parallely, the cells were exposed to DA (10^{-6}M) and to control (C) medium for 30 min (fig. 1b). Results are the mean $\pm$ sem of 4 different experiments.

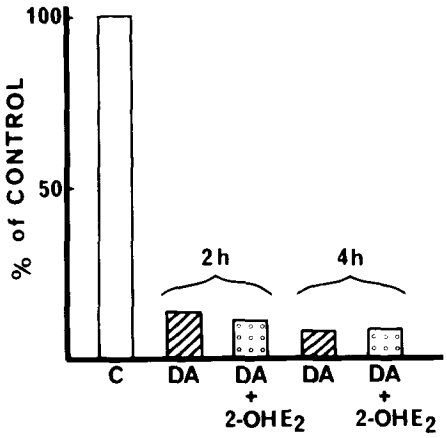

Fig. 3 - In one syringe, PRL-adenomatous cells were exposed for 2 and 4 hours (2h, 4h) to DA (10^{-6}M) alone and in another synringe running parallely, 2-OHE_2 (10^{-6}M) was added to DA (10^{-6}M). The absolute value of PRL concentration corresponding to 100 % of control is 2300 ng/ml.

submitted to DA, the inhibition of PRL-secretion (80 % of the control) was unaffected by the preexposure to these steroids and was identical to that observed with DA alone. As well the rebound effect on PRL secretion observed after DA inhibition was the same with or without preexposure of the cells to 2-OHE_2 and 17 β E_2 (figs 2 and 4). When 2-OHE_2 was added to DA, the PRL inhibition (80 % of the control) was identical to that obtained with DA alone (fig. 3).

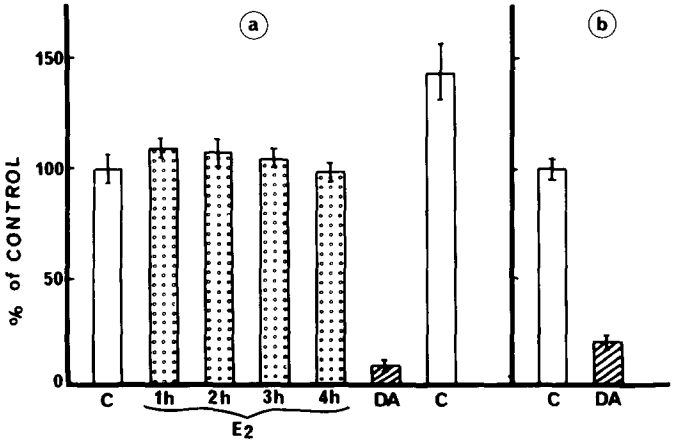

Fig. 4 - In one syringe (fig. 4a) after 1 hour of steady state (control: C), PRL-adenomatous cells were exposed for 4h to 17 βE_2 (10^{-6}M) followed by 30 min DA (10^{-6}M) and 30 min with control (C) medium. In another syringe running parallely the cells were exposed to DA (10^{-6}M) and to control (C) medium for 30 min (fig. 4b). Results are the mean of 4 different experiments.

DISCUSSION

These studies clearly demonstrate that the catecholestrogen 2-OHE_2 does not modifiy the release of PRL from superfused human PRL-adenomatous cells. Moreover 2-OHE_2 does not antagonize the PRL-inhibitory effect of dopamine (DA) on these cells. These results are in good correlation with the absence of interaction of 2-OHE_2 with dopamine receptors located on the membranes on these cells. They indicate that 2-OHE_2 does not act as a DA agonist or a DA antagonist on the PRL secretion from human PRL-secreting adenomas as it has been shown in normal humans or in animals.

Using an other catecholestrogen, 2 hydroxyestrone (2-OHE_1) which serum prolactin in normal women (10), Franks et al. have shown that this compound did not affect prolactin secretion in hyperprolactinemic women (11). Thus catecholestrogens, like 2-OHE_2 and 2-OHE_1 , do not seem to have the same effect in normal and in hyperprolactinemic subjects and these differences may be of physiopathological interest.

The failure of 2-OHE_2 to interact with DA receptors and to modify the PRL secretion from human PRL-adenomatous cells signifies that this compound does not seem to be implicated in the control of PRL secretion from prolactinomas, as it seems to be from normal hypophysis.

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