Biochemical Pharmacology, Vol. 31, No. 5, pp. 707–710, 1982.
Printed in Great Britain

0006-2952/82/050707-04 $03 00/0

INTERACTION OF ESTROGEN RECEPTORS WITH DRUGS THAT AFFECT PROLACTIN SECRETION*

YAIR LIEL,† MIRIAM MARBACH, LILIAN AFLALO, SEYMOUR M. GLICK and JOSEPH LEVY
Division of Endocrinology, Soroka Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

(*Received* 3 *June* 1981; *accepted* 9 *September* 1981)

Abstract—The competition of bromocriptine and lisuride hydrogen maleate (LIM) with estradiol binding to various tissues was evaluated by the dextran coated charcoal method.

Bromocriptine and LIM competitively inhibited the binding of [^{3}H]estradiol to its cytosolic receptors in rat uterine, pituitary and hypothalamic tissue and in DMBA induced mammary tumors. K_i was 2×10^{-5} M for bromocriptine and 2×10^{-4} M for LIM. Metoclopramide, dopamine and L-dopa had no significant effect on [^{3}H]estradiol binding. The interaction of bromocriptine and LIM was specific for estrogen receptors. There was no interaction with progesterone receptors from rat uterus and pituitary and with testosterone receptors from rat epididymis and testis.

When tested for estrogenity in the immature rat uterus, bromocriptine and LIM induced specific estrogen inducible proteins such as cytosolic estrogen and progesterone receptors. However, they do not affect the uterine/body weight ratio and peroxidase activity.

A clear interaction of inhibitors (bromocriptine and LIM) of prolactine secretion, with cytosolic estrogen receptors from various tissues was shown. Some *in vivo* estrogenic effect was also demonstrated in the immature rat uterine system.

We have previously shown that reserpine and chlorpromazine [1], which are known to induce prolactin secretion, competitively inhibited [^{3}H]estradiol binding to its cytosolic receptors and induced estrogenic markers *in vivo*. Since estrogens have a dominant role in the modulation of prolactin secretion [2–4], we decided to examine other drugs known to affect prolactin secretion for possible interaction with estrogen receptors as a possible additional mechanism for their action [5, 6]. The chemical structures of the drugs tested are shown in Fig. 1.

The results indicate that bromocriptine and lisuride (LIM) indeed interact with estrogen receptors isolated from various tissues while dopamine, L-dopa and metoclopramide do not interact with estrogen receptors.

MATERIALS AND METHODS

[2,4,6,7-^{3}H]Estradiol-17 β (85 Ci/mmole) and [1,2,6,7-^{3}H]testosterone (83 Ci/mmole) were obtained from the Radiochemical Centre, Amersham, U.K.; R5020 [17-methyl-^{3}H]17α-dimethyl-19 nor-4,9 pregnadiene-3,20-dione (80-90 Ci/mmole) was obtained from New England Nuclear (Boston, MA) and unlabelled steroids from Ikapharm (Ramat-Gan, Israel). Bromocriptine (CB-154) was a gift of Dr. Fluckiger from Sandoz Co. (Basel, Switzerland). Lisuride hydrogen maleate (LIM) was a gift of Dr. Horowski from the Schering AG (Berlin, West Germany).

In vitro *experiments*. The dextran coated charcoal technique was applied for the competitive studies of estrogen, progesterone and testosterone receptors in the cytosol of rat uterus, hypothalamus, pituitary, DMBA-induced mammary tumour and human breast cancer tissue, as previously described [7]. In short, following dissection, the tissues were cleaned and frozen and kept at −70° until the time of the assay. All procedures during the assay were done at 0–4°. The tissues were pulverized, homogenized and centrifuged for 35 min at 35,000 *g* in TED buffer (10 mM Tris–HCl, 1.5 mM EDTA and 0.5 mM dithiotreitol, pH 7.4) and the cytosol was collected. For the competition studies, the radioactive ligand (either estradiol, progesterone, testosterone or dexamethasone) in concentrations between 80–400 pmole/l was incubated for 24 hr at 4° in the absence and in the presence of various concentrations of the unlabelled competitor drugs (either bromocriptine, LIM, L-dopa, dopamine or metoclopramide) in concentrations up to 5×10^4 M. K_i was derived from Lineweaver–Burk plots.

In vivo *experiments*. Four groups of 21–23 day-old immature female rats were studied. All the animals were injected subcutaneously daily for 3–4 days with the test substance as follows: saline (C), estradiol 5μg (ED_2), bromocriptine 25μug (CB-154), and lisuride hydrogen maleate 25μg (LIM). Estradiol and LIM were dissolved in 10% ethanol and bromocriptine in 25% ethanol. The animals were killed by cervical dislocation 24 hr after the last injection. The uteri were removed, cleaned, weighed and subsequently homogenized before being tested for cytosolic estrogen and progesterone receptors as previously described [7], by the dextran coated charcoal method. Peroxidase was estimated by the guaiacol assay [8].

* Presented in part at the 2nd Innsbruck Winter Conference, 1981.

† Correspondence should be addressed to: Y. Liel, Endocrine Laboratory, Soroka Medical Center, P.O. Box 151, Beer-Sheva, Israel.

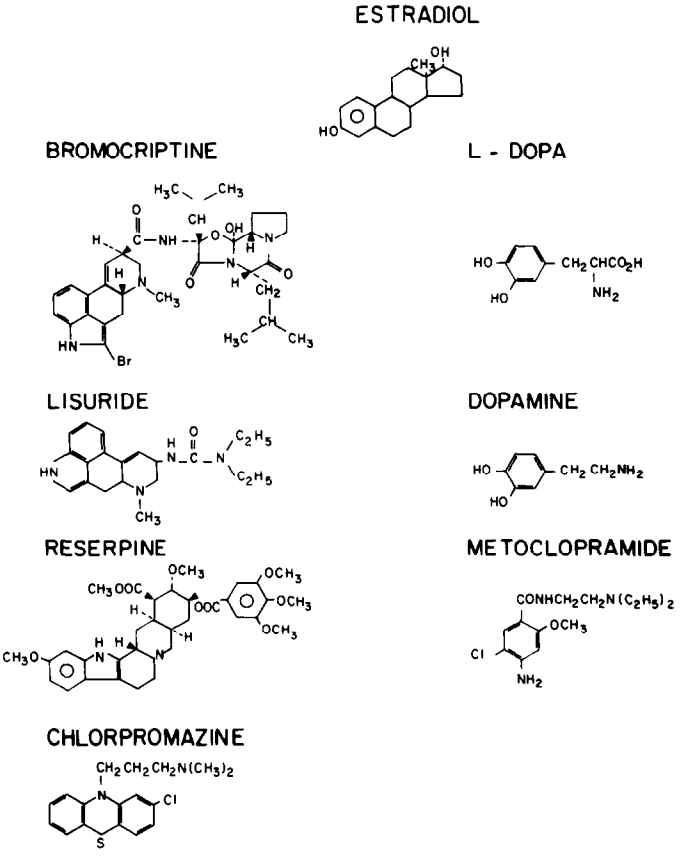

Fig. 1. Chemical structure of several drugs affecting prolactin secretion.

Statistical analysis. The statistical significance of differences between various experimental values was assessed by the Student's *t*-test.

RESULTS

Bromocriptine and LIM competitively inhibited the binding of [^{3}H]estradiol to its cytosolic receptors in rat uterine, pituitary and hypothalamic tissue and in breast tumours; DMBA induced in rats and human breast cancer (Table 1). The calculated K_i values in the rat uterus for bromocriptine was 1.4×10^{-5} M and for LIM, 1.3×10^{-4} M. A representative Lineweaver–Burk plot describing bromocriptine competitive inhibition of [^{3}H]estradiol binding to rat pituitary cytosol is presented in Fig. 2. No interaction was found between dopamine, L-dopa, metoclopramide and estrogen receptors. Lack of interaction of bromocriptine and LIM in concentrations of up

Table 1. K_i values for the competitive inhibition of CB-154 and LIM with [^{3}H]estradiol binding in cytosols from various tissues

Tissue	Kd Estradiol	K_i CB-154	K_i LIM
	$\times 10^{-10}$ M	$\times 10^{-5}$ M	$\times 10^{-4}$ M
Uterus (immature)	1.1	1.4	1.3
Pituitary	1.5	2 8	3.5
Hypothalamus	2.5	2.7	1.4
DMBA induced mammary tumor	1 8	2.0	1.5
Human breast carcinoma	1. 9	6.2	1.0

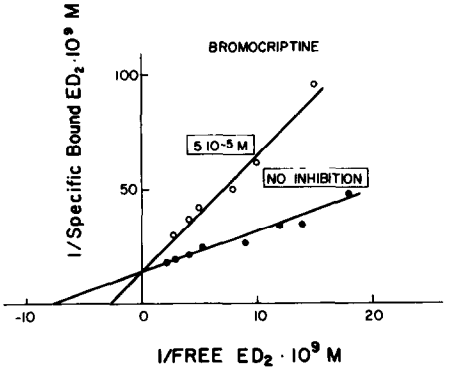

Fig. 2. Competitive inhibition by bromocriptine of specific [^{3}H]estradiol binding to rat pituitary cytosol (Lineweaver–Burk plot). Binding of tritiated estradiol in concentration of 80–400 pmole/l was measured by the dextran-coated charcoal technique in the absence of competition and in the presence of 5×10^{-5} M of bromocriptine.

Table 2. Interaction of CB-154 and LIM with different steroid receptors

RECEPTOR	TISSUE TESTED	LIGAND	KIND OF INTERACTION
Estradiol	Various	(^{3}H) Estradiol	Competitive inhibition
Progesterone	Uterus	(^{3}H) Progesterone	No interaction
	Pituitary		
	Uterus	(^{3}H) R 5020	No interaction
	Pituitary		
Testosterone	Epididymis	(^{3}H) testosterone	No interaction
	Testis		

to 5×10^{-4} M, with progesterone and testosterone receptors is summarized in Table 2.

In vivo studies (Fig. 3) revealed significant estrogenic activity of both bromocriptine and LIM as expressed by increments in uterine cytosolic estradiol and progesterone receptors. The drugs had no effect on the uterine/body weight ratio and on peroxidase activity (not shown).

DISCUSSION

The results of the present study, add to previous results from our laboratory [1], and suggest that several drugs, such as bromocriptine, LIM, reserpine and chlorpromazine, that are thought to affect prolactin secretion through the dopaminergic system, compete with estradiol for its cytosolic receptors *in vitro*. Some of these drugs induce specific estrogenic markers *in vivo*. However, several other drugs which are known to affect the dopaminergic system such as dopamine, L-dopa and metoclopramide did not interact with estrogen receptors. The K_i of interaction with the estradiol receptor was relatively low, but the interaction with estrogen receptors was found to be specific, as indicated by the lack of interaction with receptors for progesterone and testosterone.

It is of interest that of the drugs that were found to interact with estrogen receptors, only reserpine induced an increase in uterine/body weight ratio and peroxidase activity [1]. Bromocriptine and LIM increased only estradiol receptors and progesterone receptors significantly. Moreover, only reserpine exerted an anti-estrogenic effect *in vivo* when injected concomitantly with estradiol [1].

Pharmacokinetic studies of bromocriptine in man [9] and rat [10] revealed peak plasma concentrations in the nmolar range, significantly lower than the concentrations of the drugs used in our *in vitro* studies. However, bromocriptine has been shown to concentrate in the pituitary [10]. If drugs of this type were to be concentrated in various target organs this phenomenon might help explain the unequivocal *in vivo* activation of markers believed to be specific for estrogenic activity, in the rat uterus, as exemplified by increments in both estrogen and progesterone receptors. Though metabolites of either bromocriptine or LIM could act as stronger estrogenic compounds *in vivo*, the data about such derivatives are too scant to accept this possibility.

The present study suggests a direct interaction with the estradiol receptor system of various drugs which affect the dopaminergic system. Recently a reciprocal interaction of estradiol with the dopaminergic system was demonstrated under *in vitro* conditions [11]. Dopamine was much less effective in suppressing the release of prolactin from anterior pituitary tissue of estrogen-treated rats than from tissue of the controls. The estrogen treatment reduced the capacity of prolactin cells to incorporate dopamine into prolactin secretory granules.

The drugs tested included both known dopaminergic agonists, which are prolactin depressors (dopamine, L-dopa, bromocriptine and LIM) and dopaminergic antagonists which are prolactin stimulators (reserpine, chlorpromazine, metoclopramide). However, there seemed to be no relation between the mode of action of the drugs in the dopaminergic system and its estrogenic properties.

Though the pharmacological significance of these results is still unclear, it is suggested that the specific interaction of bromocriptine and LIM, with estrogen receptors in the hypothalamus and pituitary, may have a role in the mechanism of action of these drugs with respect to prolactin secretion.

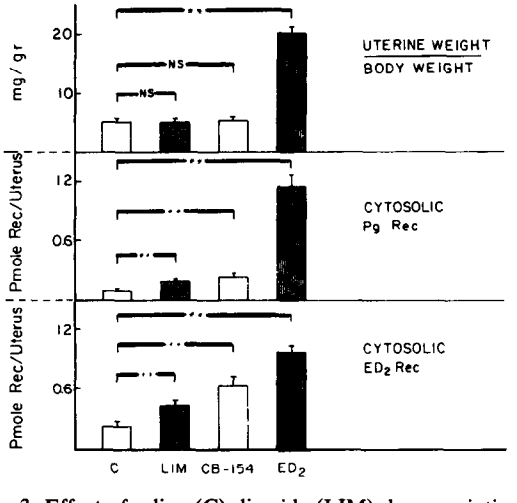

Fig. 3. Effect of saline (C), lisuride (LIM), bromocriptine (CB-154) and estradiol (ED_2) on uterine/body weight ratio, cytosolic progesterone and estradiol receptor in the immature rat uterus. Values are means ± S.E.M. The number of individual observations was between 9 and 23 **P = 0.05, NS = not significant.

REFERENCES

1. J. Levy, A. Burshell, M. Marbach, L. Aflalo and S. M. Glick, in *Pharmacological Modulation of Steroid Action* (Eds E. Genazzani, F. DiCarlo and W. P. Mainwaring), p. 111. Raven Press, New York (1980).
2. S. S. C. Yen, Y. Ehara and T. M. Siler, *J. clin. Invest.* **53**, 652 (1974).
3. C. L. Chen and J. Meites, *Endocrinology* **86**, 503 (1970).
4. E. Haug and K. M. Gautvik, *Endocrinology* **99**, 1482 (1976).
5. J. W. Kebabian, G. L. Petzold and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **69**, 2145 (1972).
6. P. Falaschi, G. Frajese, F. Sciarra, A. Rocco and C. Conti, *Clin. Endocr.* **8**, 427 (1978).

7. J. Levy, A. Burshell, M. Marbach, L. Aflalo and S. M. Glick, *J. Endocr.* **84**, 371 (1980).
8. C. R. Lyttle and E. R. DeSombre, *Nature, Lond.* **268**, 337 (1977).
9. H. F. Schran, I. B. Suryakant, N. J. Schwarz and L. J. Loeffler, *Clin. Chem.* **25**, 1928 (1979).
10. E. Flückiger, in *Bromocriptine—A Clinical and Pharmacological Review* (Eds M. O. Thorner, E. Flückiger and D. B. Calne), p. 42. Raven Press, New York (1980).
11. G. A. Gudelsky, D. D. Nansel and J. C. Porter, *Endocrinology* **108**, 440 (1981).