Biochemical Pharmacology Vol. 20, pp. 547–552, Pergamon Press, 1971. Printed in Great Britain

CHLORPROMAZINE INHIBITION OF TRYPTOPHAN OXYGENASE

J. MØRLAND

Department of Pharmacology, University of Oslo, Blindern, Oslo 3, Norway

(*Received* 23 *June* 1970; *accepted* 7 *August* 1970)

Abstract—The effect of chlorpromazine on the activity of liver tryptophan oxygenase was examined in rat liver homogenates and in perfused rat livers. Chlorpromazine was found to inhibit the enzyme in the assay system (50 per cent inhibition at $3 \cdot 10^{-4}$M). The tryptophan oxygenase activity in perfused livers, however, was not decreased by perfusing with 10^{-4}M chlorpromazine for 3 hr. A slight decrease was found in livers perfused with $4 \cdot 10^{-4}$M for 3 hr. Chlorpromazine did not interfere with the steroid induction of the enzyme. This indicates that the effect of dexamethasone on protein synthesis in the liver does not depend on any of the membrane functions which are influenced by the membrane stabilizing effect of chlorpromazine.

CHLORPROMAZINE stabilizes erythrocytes against hypotonic and mechanical disruption.[1] Chlorpromazine is also a membrane stabilizer as defined by Shanes[2, 3] reducing cell excitability by interfering with ion fluxes across cell membranes.[4] Recently Wolff and Jones[5] showed that chlorpromazine in membrane stabilizing concentrations inhibits hormone activation of adenyl cyclase in thyroid, adrenal and liver tissue without interfering with the basal activity of the enzyme. Studies on the isolated heart in this laboratory indicate that chlorpromazine possibly interferes with a step between the hormone receptor and adenyl cyclase,* both located in the cell membrane. Furthermore, it has been shown that chlorpromazine and related membrane stabilizing agents interfere with other membrane enzymes and constituents.[6–8] These effects of chlorpromazine are apparently unspecific and closely related to the general membrane stabilizing action. It is therefore likely that chlorpromazine will prevent hormone actions, which depend on completely undisturbed cell membrane functions. Chlorpromazine might thus be useful as a tool for examining whether a particular hormone acts through adenyl cyclase or another "second messenger" system associated with the cell membrane.

Indirect evidence for chlorpromazine inhibition of steroid induced synthesis of tryptophan oxygenase [L-tryptophan: oxygen oxidoreductase (EC 1.13.1.12)] exists.[9] Corticosteroids affect cell membranes[10] but the relationship between this effect and their ability to regulate protein synthesis is unknown. The present work was initiated in order to examine if chlorpromazine could interfere through membrane stabilization with tryptophan oxygenase induction by dexamethasone. The results, however, gave no evidence supporting this possibility. On the contrary, chlorpromazine in membrane stabilizing concentrations exerted a direct inhibition of tryptophan oxygenase in the assay system.

* A. LANGSLET, *Europ. J. Pharmac.*, submitted for publication.

MATERIALS AND METHODS

Liver perfusion

Male Wistar rats (300–340 g) were maintained on standard laboratory rat chow and fasted 18 hr prior to experiments. Livers were isolated and perfused by a technique described previously[11] with the following modification: During the procedure for isolation of the liver, the polyethylene cannula inserted into vena porta was connected to an infusion pump, infusing oxygenated perfusate at a rate of 5 ml/min. This perfusate was not recirculated. In this way the portal circulation was shut off for only a few seconds. The preparation was then transferred to the circulation unit. The volume of recirculating perfusate was about 30 ml (in addition to the small volume contained in the liver), and consisted of one part whole rat blood and two parts of buffer enriched with albumine (2·5% w/v). Liver specimens for enzyme analysis were taken at the beginning of each perfusion and at times indicated. Chlorpromazine was added to the perfusate after the first specimen was taken, and dexamethasone was added 30 min after chlorpromazine.

The experiments were conducted in the dark to reduce the photooxidation of chlorpromazine during perfusion.

Tryptophan oxygenase assay

Tryptophan oxygenase was measured by the procedure of Seglen and Jervell.[12] The enzyme activity was calculated as μmoles of kynurenine formed per hour at 37° per gram of liver tissue (wet weight) and expressed here as per cent of values obtained at zero time (perfusion experiments) or as per cent of controls (homogenate experiments). Assay supernatants were examined for *N*-formylkynurenine and kynurenine metabolites by the methods of Mehler and Knox,[13] and Jago, Nelson and Rose.[14]

Chemical agents

Chlorpromazine (Largactil, Pharma Rhodia) was used with appropriate control solution. Dexamethasone phosphate (Decadron, Merck, Sharp and Dohme) was a gift from Apotekernes Laboratorium, Oslo, Norway. Albumin (Bovine fraction V) was purchased from Sigma. Other chemicals were reagent grade.

RESULTS

Effect of chlorpromazine on tryptophan oxygenase in vitro

The effect of chlorpromazine on tryptophan oxygenase *in vitro* is shown in Fig. 1. As seen from the figure 50 per cent inhibition was obtained at about $3 \cdot 10^{-4}$M and near full inhibition at $2 \cdot 10^{-3}$M when chlorpromazine was added to the tryptophan oxygenase assay mixture.

This enzyme assay is based upon the spectrophotometrical measurement of kynurenine at 365 nm. One metabolic pathway for tryptophan is shown in Fig. 2. To make sure that the measured fall in kynurenine production was not due to either inhibition of formamidase (Aryl-formylamine amidohydrolase EC 3.5.1.9) or increased rate of the further metabolism of kynurenine—the assay supernatant from treated livers was examined for *N*-formylkynurenine and "post"-kynurenine metabolites. These metabolites did not increase. Dexamethasone (6–600 μg) added directly to the tryptophan oxygenase assay mixture did not interfere with the inhibitory action of chlorpromazine.

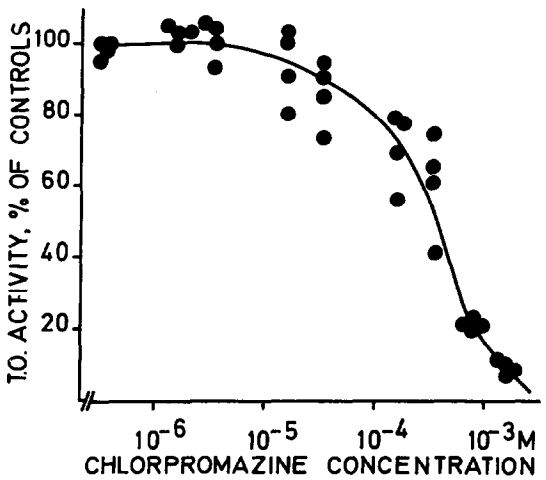

FIG. 1. The effect of chlorpromazine on tryptophan oxygenase activity *in vitro*. Homogenates from four rats, fasted for 18 hr, were prepared. Three portions of 20 ml of each homogenate served as controls. Chlorpromazine was added to other portions of homogenate from the same rat, to obtain the indicated concentrations. The results are expressed in per cent of control values. Each point represents the mean of two aliquots from the same portion of homogenate.

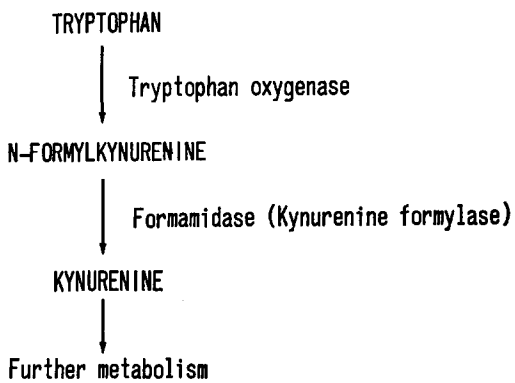

FIG. 2. One metabolic pathway for tryptophan in rat liver.

Effect of chlorpromazine on tryptophan oxygenase in the perfused liver

A significant loss of liver tryptophan oxygenase activity occurred after perfusion for 3 hr with $4 \cdot 10^{-4}$M chlorpromazine. Lower concentrations did not affect the enzyme activity (Fig. 3). The development of this loss during the perfusion is shown in Fig. 4. In five perfusions without any additions the tryptophan oxygenase activity remained constant (within $\pm$ 15%) for at least 5 hr.

Interference of chlorpromazine with steroid induction

Chlorpromazine was added to the perfusate at concentrations (10^{-4}M or $4 \cdot 10^{-5}$M) that did not interfere with the basal activity of tryptophan oxygenase. Dexamethasone was added after 30 min. The results are shown in Fig. 5. (In single experiments

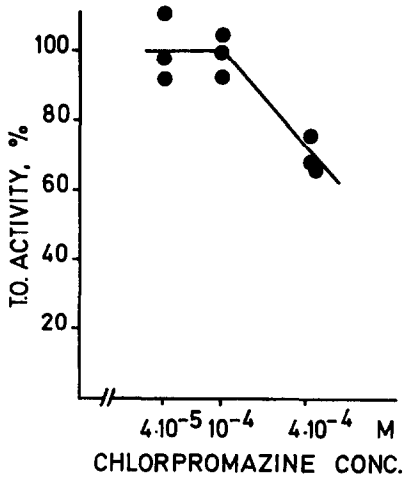

FIG. 3. Tryptophan oxygenase activity in livers perfused with chlorpromazine for 3 hr. The chlorpromazine concentration given is that at the beginning of the perfusion. Tryptophan oxygenase activity is expressed as per cent of the activity at zero time. Each point represents the mean of two or three liver samples from one perfusion experiment (range less ± 5%).

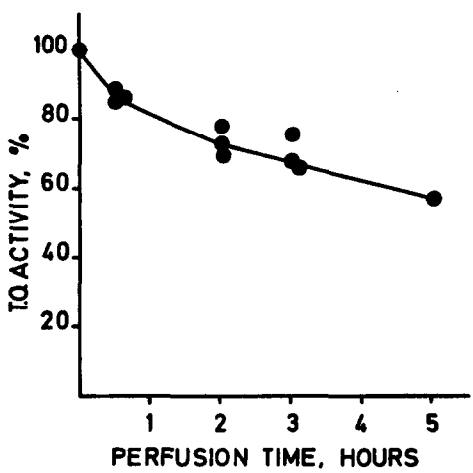

FIG. 4. The effect of $4 \cdot 10^{-4}$M chlorpromazine on the tryptophan oxygenase activity during liver perfusion. Three experiments were performed. Tryptophan oxygenase activity is expressed as per cent of the activity at the beginning of the perfusion. Each point represents the mean of two or three liver samples from one perfusion experiment (range less than ± 5%).

also lower concentrations were used, 10^{-5}M and $5 \cdot 10^{-6}$M. The results were the same as those obtained with 10^{-4}M or $4 \cdot 10^{-5}$M.) It is concluded that chlorpromazine in concentrations known to stabilize cell membrane did not interfere with enzyme induction caused by dexamethasone.

DISCUSSION

Inhibition of tryptophan oxygenase by chlorpromazine

Tryptophan oxygenase, an enzyme of the cell sap, was inhibited *in vitro* by addition of chlorpromazine (10^{-5}M and higher). From other studies chlorpromazine in con-

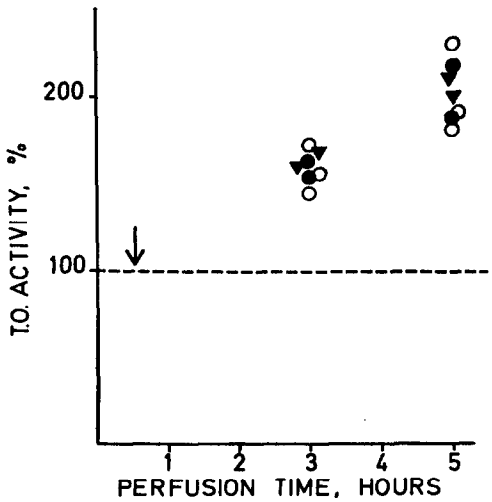

FIG. 5. Steroid induction of tryptophan oxygenase. Dexamethasone (20 mg/ml) was always given at 30 min (↓). Three experiments were carried out without further additions (○), in addition to dexamethasone two livers received chlorpromazine (10^{-4}M) after 3 min perfusion (●), and two chlorpromazine ($4 \cdot 10^{-5}$) (▼). Tryptophan oxygenase activity is expressed as per cent of the zero time activity. Each point represents the mean value of two or three liver samples from one perfusion experiment (range less than ± 5%). The dotted line indicates the values of the control perfusions (perfusions without any additions).

centrations 10^{-5}M–10^{-3}M is known as an inhibitor of several enzymes;[6, 15, 16] most of these are associated with the cell membrane.

In liver perfusions rather high concentrations of chlorpromazine 10^{-4}M, did not affect the basal level of tryptophan oxygenase. At $4 \cdot 10^{-4}$M a loss of enzyme activity was found. As the assay was carried out after diluting the samples by a factor of a hundred, this inhibition cannot be explained by the presence of chlorpromazine in the assay system. Chlorpromazine is metabolized in the liver and the possibility that the inhibitory effect is due to metabolites cannot be ruled out. A more remote explanation might be that chlorpromazine in spite of this metabolism was concentrated in the liver during the perfusion, and thus might reach inhibiting concentrations in the assay (diluted 1:100).

Steroid induction of tryptophan oxygenase in the presence of chlorpromazine

The concentrations of chlorpromazine used in these experiments are known to inhibit a variety of membrane functions including hormone activation of adenyl cyclase and the activity of other membrane-bound enzymes. This inhibitory action is, as mentioned earlier, unspecific and related to membrane stabilization. It is unlikely that hormone effects involving membrane receptors and the production of a "second messenger" at this level should remain completely unaffected when chlorpromazine is present.

In these experiments no specific inhibition of the dexamethasone induced increase of tryptophan oxygenase was found. These results are therefore suggestive of basically different mechanisms of action for steroid hormones and for hormones activating adenyl cyclase. Dexamethasone, furthermore, did not depend on the completely

normal functional state of the cell membrane to accomplish its induction of tryptophan oxygenase in the liver. This is in agreement with the current views concerning steroid mechanism of action, although other cellular membranes known to be affected by chlorpromazine, have been discussed as steroid targets.[17–20]

Chlorpromazine action on tryptophan oxygenase in vivo

The results are not in disagreement with the observations of many authors,[9, 21–23] who show that chlorpromazine administration to the intact animal increase tryptophan oxygenase activity, as this effect is supposed to be due to endogenous release of ACTH provoked by chlorpromazine.[21, 24]

The discrepancy between the results reported here and those of Kunz *et al.*[9] who indirectly found that chlorpromazine might inhibit the steroid induction of tryptophan oxygenase, may be due to differences in experimental conditions (Kunz *et al.*[9] using intact animals and endogenous steroids) and different assay conditions for measuring tryptophan oxygenase.

Acknowledgements—This investigation was supported by research grants from the Norwegian Council for Science and the Humanities (NAVF). The technical assistance of Mrs. Turid Gangnaes, Miss Kirsten Garberg and Miss Tove Nordstad is gratefully acknowledged. Finally, I want to thank Dr. Ivar Øye for helpful discussions.

REFERENCES

1. P. M. SEEMAN, *Int. Rev. Neurobiol.* **9**, 145 (1966).
2. A. M. SHANES, *Pharmac. Rev.* **10**, 59 (1958).
3. A. M. SHANES, *Pharmac. Rev.* **10**, 165 (1958).
4. A. LANGSLET and I. ØYE, *Nord. Med.* **83**, 553 (1970).
5. J. WOLFF and A. B. JONES, *Proc. natn. Acad. Sci. U.S.A.* **65**, 454 (1970).
6. I. JÄRNEFELT, *Biochim. biophys. Acta* **59**, 643 (1962).
7. I. D. ROBINSON, I. LOWINGER and B. BETTINGER, *Biochem. Pharmac.* **17**, 1113 (1968).
8. W. L. HUBBELL, H. M. MCCONNELL and J. C. METCALFE, *Br. J. Pharmac.* **35**, 374P (1969).
9. K. KUNZ, Z. SIMANE and M. VOJTECHOVSKY, *Europ. J. Pharmac.* **3**, 87 (1968).
10. P. M. SEEMAN, *Biochem. Pharmac.* **15**, 1632 (1966).
11. P. O. SEGLEN and K. F. JERVELL, *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 308 (1969).
12. P. O. SEGLEN and K. F. JERVELL, *Biochim. biophys. Acta* **171**, 47 (1969).
13. A. H. MEHLER and W. E. KNOX, *J. biol. Chem.* **187**, 431 (1950).
14. M. V. JAGO, J. F. NELSON and S. ROSE, *Biochim. biophys. Acta* **67**, 513 (1963).
15. L. B. KHOUW, T. N. BURBRIDGE and V. C. SUTHERLAND, *Biochim. biophys. Acta* **73**, 173 (1963).
16. P. LINGJAERDE, *T. norske Laegefor.* (The Journal of the Norwegian Medical Association) **87**, 1401 (1967).
17. H. C. PITOT, H. S. CHO and C. LAMAR, *J. Cell comp. Physiol.* **66**, suppl. 1, 163 (1965).
18. D. W. JAMES, B. R. RABIN and D. J. WILLIAMS, *Nature, Lond.* **224**, 371 (1969).
19. R. F. COX and A. P. MATHIAS, *Biochem. J.* **155**, 777 (1969).
20. G. WEISSMANN, in *Lysosomes in Biology and Pathology* (Eds. J. T. DINGLE and H. B. FELL) in *Frontiers of Biology* (Eds. A. NEUBERGER and E. L. TATUM), p. 276, North-Holland, Amsterdam (1969).
21. R. L. SMITH, R. P. MAICKEL and B. B. BRODIE, *J. Pharmac. exp. Ther.* **139**, 185 (1963).
22. I. KUSCH and I. HEINRICH, *Acta biol. med. germ.* **10**, 554 (1963).
23. I. NOMURA, *Endocrinology* **76**, 1190 (1965).
24. M. HOLZBAUER and M. VOGT, *Br. J. Pharmac.* **9**, 402 (1954).