Biochemical Pharmacology, Vol. 35, No. 11, pp. 1899–1902, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

CHRONIC EXPOSURE OF HUMAN CELLS IN CULTURE TO THE TRICYCLIC ANTIDEPRESSANT DESIPRAMINE REDUCES THE NUMBER OF BETA-ADRENOCEPTORS

ULRICH E. HONEGGER,*† BEAT DISLER* and ULRICH N. WIESMANN‡
* Department of Pharmacology and ‡ Department of Pediatrics, University of Bern, 3010 Bern, Switzerland

(*Received* 8 *August* 1985; *accepted* 6 *December* 1985)

Abstract—The effects of the antidepressant drug desipramine (DMI) on the density of beta-adrenoceptor sites were studied on intact cultured human cells: skin fibroblasts, lung fibroblasts and macrophages.

Direct binding studies were performed with the radioligand ^{3}H-CGP 12177, a hydrophilic beta-adrenergic antagonist. The confluent cell cultures were exposed to DMI and all three cell types showed a dose-dependent decrease in the number of beta-adrenergic binding sites. This receptor desensitisation was only seen after chronic exposure of the cells to DMI. The extent of desensitisation was comparable to that seen in brain following chronic treatment of rats with DMI. The affinity of the binding sites to the radioligand was not affected by the antidepressant drug action. From these results we suggest that the *in vivo* effect of antidepressant drugs on postsynaptic beta-adrenoceptor density, at least in part, reflects a primary drug action and not only an adaptive change to presynaptic events.

In vivo effects of chronic treatments with antidepressants include changes in the density of neurotransmitter receptors in the CNS [1, 2]. In particular desensitisation of beta-adrenoceptors has been reported in rat brain [3, 4] and also in peripheral organs [4].

Diminished beta-adrenoceptor densities have not only been noted after treatment with classical tricyclic drugs, with atypical or MAO-inhibitor-type antidepressants but also following electroconvulsive treatment [5]. In addition to the reduced beta-adrenoceptor number a diminished cAMP-response to noradrenaline has been described in brain slices after chronic treatment of rats with antidepressants [6]. This underlines the physiological significance of the drug-induced receptor desensitisation.

The reduction in the number of postsynaptic beta-adrenoceptors could be secondary to presynaptic events which result in an increased neurotransmitter concentration at the receptor sites. Receptor desensitisation, however, could also be a consequence of primary changes in the postsynaptic membrane, e.g. alterations of the membrane phospholipid matrix. The difficulties in distinguishing between pre- and postsynaptic drug actions resulting in beta-receptor desensitisation in brain and peripheral organs prompted us to use cultured human cells as a simple model for "postsynaptic" cells. Fibroblasts and macrophages possess a functional beta-adrenergic system [7, 8]. We have recently been able to show that chronic exposure of human skin fibroblasts to the tricyclic antidepressant desipramine (DMI) induces changes in phospholipid metabolism [9]. Such changes could be due to the lysosomotropic action of DMI [10], which inhibits lysosomal phospholipid degradation.

Alterations in the membrane phospholipid composition can modulate the coupling of beta-adrenoceptors to the adenylate cyclase and hence the number of functional receptor sites. Very recently McOsker *et al.* have reported that the incorporation of phosphatidylinositol into the plasmamembrane of turkey erythrocytes by means of specific phospholipid-transfer-proteins led to a reduction of isoproterenol-stimulated cAMP-formation [11]. Phosphatidylinositol and to a lesser degree phosphatidylethanolamine showed an overproportionate increase after exposure of cultured fibroblasts to DMI [9]. In particular phosphatidylinositol appears to play a prominent role in some receptor regulated neurotransmitter effects [12].

MATERIALS AND METHODS

Cell cultures

Three different human cell-types were used.

(1) *Skin fibroblasts.* Skin biopsies for fibroblast cultures were obtained after informed consent from healthy volunteers undergoing minor surgery. The methods for establishing and maintaining fibroblast cultures have been previously described [13].

(2) *MRC-5 fibroblasts.* Fetal lung fibroblasts at the 10th cell passage were obtained from the British Medical Council (U.K.).

(3) *Macrophages.* These cells were cultured from a spontaneously transformed monocyte/macrophage cell line that was obtained from peripheral blood monocytes in our laboratory. The cells, which are tetraploid, grow attached to the plastic culture material and are partially contact inhibited. They secrete JL-1 and phagocytose latex-particles.

† Author to whom all correspondence should be addressed: Dr. U. Honegger, Dept. of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3010 Bern, Switzerland.

Stock cultures were maintained as monolayers in 75 cm^2 Falcon tissue culture flasks. The cultures were incubated in Eagle minimum essential medium supplemented with 10% fetal calf serum, nonessential amino acids, penicillin (100 U/ml) and chlortetracyclin (15 μg/ml). The medium which was changed twice a week was buffered at pH 7.4 with 15 mM $NaHCO_3$ and 5% CO_2 in air. The stock cultures were grown to confluency at 37° in a CO_2-incubator. Two confluent 75 cm^2 flask cultures were used to start one 850 cm^2 roller bottle (Falcon) culture. Prior to the binding study, cell cultures were washed *in situ* with Hank's solution and were then scraped off into 100 ml of Hank's solution per roller bottle. The resulting suspensions were centrifuged at 500 *g* for 10 min. The pellets were washed with 5 ml of Hank's solution and centrifuged as described above. The pellets were pooled and resuspended in Hank's solution to give a final concentration of about 3 mg of cell protein per ml. Immediately before the binding assay the cells were resuspended by repeated gentle pipetting.

Binding assay

Receptor binding studies on intact cultured cells were performed as previously described [14]. Two-hundred-microlitre aliquots of the cell suspension containing about 10^6 cells were pipetted into the assay tubes. Incubations were initiated by adding 50 μl of ligand solution. The final incubation mixture contained between 0.15 and 6.0 nM of the radiolabelled compound ^{3}H-CGP 12177 [4-(3-tert. butylamino - 2 - hydroxypropoxy) - benzimidazole - 2 - on hydrochloride] a specific hydrophilic beta-adrenoceptor blocking agent [15, 16]. Nonspecific binding was determined in the presence of 1 μM timolol, a potent beta-adrenoceptor antagonist. This concentration of timolol was sufficient to displace specific binding of the labelled CGP 12177 without interfering with nonspecific binding. The tubes were constantly shaken in a water bath at 37° for 60 min. After this time steady-state binding conditions were reached. Incubations were stopped by adding 1 ml of ice-cold Hank's solution to the samples. 1.1 ml of the stopped incubation mixture were immediately withdrawn and filtered under controlled vacuum pressure through presoaked glassfiber filters (Whatman GF/C). The filters were rinsed 3 times with 5 ml of saline, dried and placed in counter vials. Cells were digested over night with Protosol (New England Nuclear). Radioactivity was determined after neutralisation and addition of 10 ml of a liquid scintillation cocktail in a Kontron MR 300 liquid scintillation counter (Kontron, Switzerland).

Direct binding studies on rat brain tissue have been carried out with the radioligand ^{3}H-dihydroalprenolol (^{3}H-DHA), a potent beta-adrenergic antagonist. The crude membrane preparation of rat cerebrum was incubated with increasing concentrations of ^{3}H-DHA (New England Nuclear) with and without 1 μM timolol to determine nonspecific binding. Except for minor modifications, tissue preparations and binding conditions were as described by Banerjee *et al.* [3].

All chemicals used were of analytical grade. Tissue culture medium and fetal calf serum were obtained from Bio AG/Gibco Europe (Switzerland). ^{3}H-CGP 12177 was kindly provided by Ciba-Geigy AG Basel (Switzerland).

RESULTS AND DISCUSSION

Figures 1 and 2 represent the data for typical equilibrium binding experiments with ^{3}H-CGP 12177

Table 1. Effects of chronic desipramine-exposure on maximal binding capacities (b_{max}) and equilibrium dissociation constants (K_D) of beta-adrenergic binding sites on cultured human cells and in rat brain

Cells/Tissue	b_{max} (fmoles/mg protein)			K_D (nM)		
	Controls	Desipramine exposed 2 μM	5 μM	Controls	Desipramine exposed 2 μM	5 μM
(A)						
Skin fibroblasts*	6.0 ± 0.4	3.8 ± 0.3‖	2.1 ± 0.4‖	0.35 ± 0.02	0.34 ± 0.04	0.38 ± 0.02
Lung fibroblasts†	9.3 ± 0.2	8.6 ± 0.5¶	5.7 ± 0.9‖	0.52 ± 0.06	0.62 ± 0.16	0.59 ± 0.20
Macrophages‡	49.0 ± 4.2	38.7 ± 6.7‖	28.5 ± 4.7‖	1.73 ± 0.49	1.80 ± 0.12	1.70 ± 0.17
		10 mg/kg.d			10 mg/kg.d	
(B)						
Rat brain§	92.0 ± 8.7	65.1 ± 8.9‖		2.62 ± 0.73	2.59 ± 0.83	

(A) Cultured cells were exposed to 0 (controls), 2 or 5 μM DMI for 12 days. ^{3}H-CGP 12177 was used as radioligand.
(B) Rats were treated with daily i.p.-injections of 10 mg DMI/kg for 6 weeks. A crude membrane fraction of the cerebrum was prepared for the binding assay. ^{3}H-dihydroalprenolol was used as radioligand.
* Values represent mean ± S.D. of 10 experiments.
† Values represent mean ± S.D. of 3 experiments.
‡ Values represent mean ± S.D. of 6 experiments.
§ Values represent mean ± S.D. of 3 experiments and a total of 15 animals per treatment group.
‖ Significantly different from controls, $P < 0.01$.
¶ Significantly different from controls, $P < 0.02$ (Student's test).

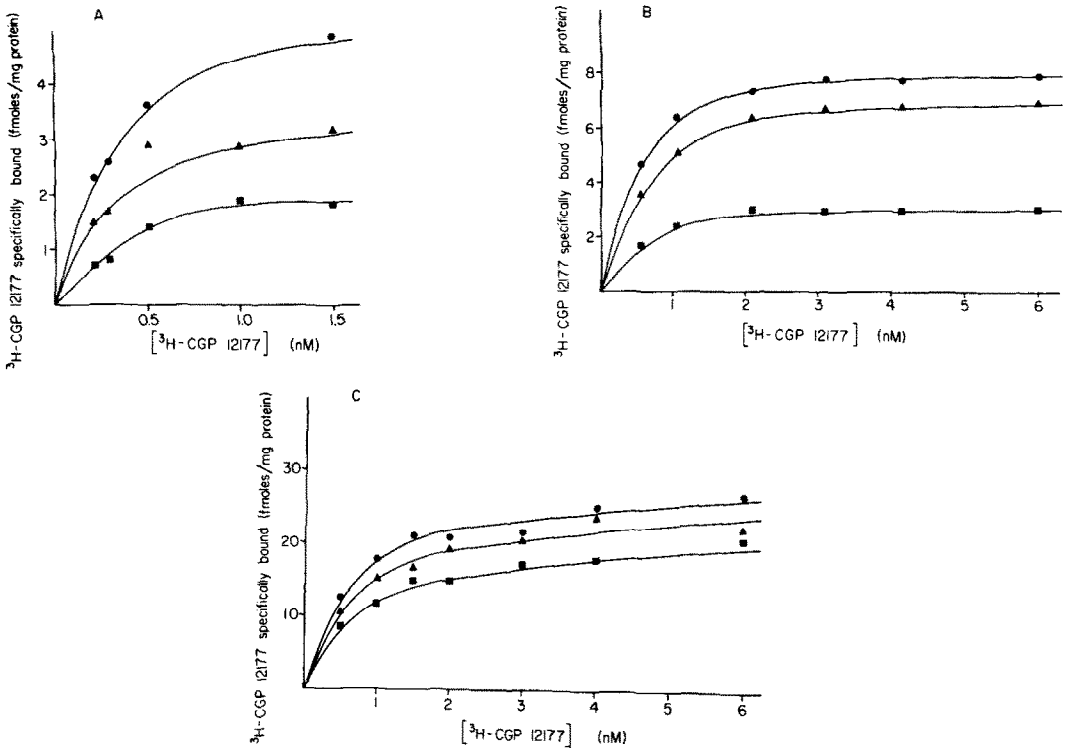

Fig. 1. Effects of desipramine on equilibrium binding of H-CGP 12177 to intact, cultured human cells. Confluent monolayer cultures were incubated in the absence or presence of desipramine (2 and 5 μM) for 12 days. Binding assays were performed with suspensions of scraped cells. Nonspecific binding was determined in the presence of 1 μM timolol. The assays were made in triplicates: (●) control cells; (▲) desipramine 2 μM; (■) desipramine 5 μM. The experimental points were fitted by nonlinear least squares regression lines. (A) Skin fibroblasts; (B) Lung fibroblasts; (C) Macrophages.

in cell culture studies and for ^{3}H-DHA in brain tissue assays. In all systems tested, specific binding to beta-adrenoceptor-sites was saturable indicating a finite number of binding sites. Values for maximal binding (b_{max}) and for equilibrium dissociation constants (K_D) were calculated by means of a modfit-program [17] written in basic and run on a Hewlett-Packard-9816 computer. The results are summarized in Table 1.

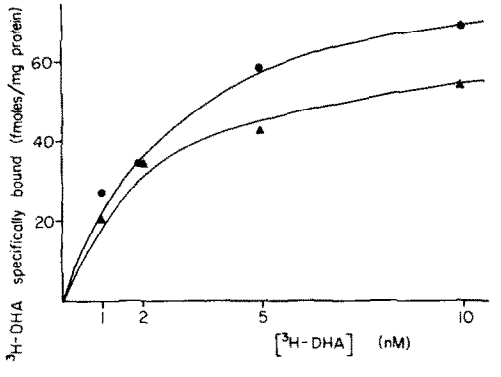

Fig. 2. Effects of chronic desipramine-treatment on equilibrium binding of ^{3}H-DHA to a crude membrane preparation of rat cerebrum. Sprague–Dawley rats were treated with daily i.p.-injections of saline (controls) or desipramine (10 mg/kg) for 6 weeks. The animals were killed by decapitation 24 hours after the last injection and the brain was quickly removed. Binding assays on crude membrane preparations were performed in quintuplicates. Non-specific binding was determined in presence of 1 μM timolol: (●) controls; (▲) desipramine-treated.

The use of the hydrophilic radioligand ^{3}H-CGP 12177 allowed us to determine beta-adrenoceptors on intact cells. In contrast to lipophilic beta-adrenergic antagonists. ^{3}H-CGP 12177 was not taken up into the cells and nonspecific binding was relatively low. The displacement of ^{3}H-CGP 12177 was stereospecific since the (−)-isomer of isoproterenol was more than two orders of magnitude more potent in displacing ^{3}H-CGP 12177 than the respective (+)-isomer (results not shown).

b_{max}-Values of control cells yielded the following beta-adrenoceptor densities: ~3000 sites per skin-fibroblast; ~5300 sites per lungfibroblast and ~13000 sites per macrophage.

Chronic exposure of cultured cells to DMI induced a decrease in the number of beta-adrenoceptor binding sites. Confluent cultures were exposed to 2.0 or 5.0 μM concentrations of DMI for 12 days. During this period the incubation medium containing the drug was changed three times. At the end of the treatment the medium was removed and the cells were suspended as described in the Methods.

The similarity of K_D-values indicates that the DMI-induced reduction in binding capacity was not accompanied by any significant alteration in binding affinity (Table 1). The addition of up to 10 μM DMI to the binding assay did not affect ^{3}H-CGP 12177-binding. This suggested that the drug itself did not directly interact with the receptor binding sites.

Drug induced receptor desensitisation required a prolonged exposure of the cells to DMI, since single short term (2 hr) incubations had no effect on beta-adrenoceptor binding. The need for chronic drug exposure and the extent of the reduction in receptor number observed in our *in vitro* model systems are comparable to *in vivo* findings [3, 4].

Our *in vitro* findings are, however, at variance with the *in vivo* observation which showed the necessity of a functional noradrenaline release for an antidepressant-induced beta-adrenoceptor desensitisation [18]. Noradrenaline is not considered to be present in cell cultures. Yet, Dibner and Insel [19] have found that fetal serum, a component of the culture medium, contains variable but measurable amounts of noradrenaline; thus its presence and its potential effectiveness on cultured cells cannot be ruled out.

A downregulation of the beta-adrenoceptor mediated cAMP-response has also been observed in cultured astroglia after chronic exposure to the antidepressant drug amitriptyline [20]. From this we conclude that antidepressant drugs act on postsynaptic beta-adrenergic transmission. This could result from an influence on receptor turnover or from a change of the receptor environment in the phospholipid bilayer. Thus, our findings support the notion that the decreased number of functional beta-adrenoceptors may, at least in part, result from drug-induced events at the postsynaptic membrane itself.

Acknowledgements—The present study was supported by the Swiss National Science Foundation and by the "Fritz Hoffmann-La Roche Stiftung". The authors would like to thank Ms. S. Probst and Ms. B. Blanchard for their excellent technical assistance.

REFERENCES

1. M. F. Sugrue, *Pharmac. Ther.* **13**, 219 (1981).
2. S. J. Peroutka and S. H. Snyder, *Science* **210**, 88 (1980).
3. S. P. Banerjee, L. S. Kung, S. J. Riggi and S. K. Chanda, *Nature, Lond.* **268**, 455 (1977).
4. U. E. Honegger and M. H. Bickel, in *Phenothiazines and Structurally Related Drugs*, Vol. 7. (Eds. Usdin, Eckert and Forrest), p. 245. Elsevier/North Holland, Amsterdam (1980).
5. D. A. Bergstrom and K. J. Kellar, *Nature, Lond.* **278**, 464 (1979).
6. F. Sulser, J. Vetulani and P. L. Mobley, *Biochem. Pharmac.* **27**, 257 (1978).
7. M. Buchwald, *Proc. natn. Acad. Sci. U.S.A.* **73**, 2899 (1976).
8. A. A. Roscher, A. J. Schmid, B. Hadorn and U. N. Wiesmann, *Pediat. Res.* **14**, 261 (1980).
9. R. Fauster, U. E. Honegger and U. N. Wiesmann, *Biochem. Pharmac.* **32**, 1737 (1983).
10. U. E. Honegger, A. A. Roscher and U. N. Wiesmann, *J. Pharmac. exp. Ther.* **225**, 436 (1983).
11. C. C. McOsker, G. A. Weiland and D. B. Zilversmit, *J. biol. Chem.* **258**, 13017 (1983).
12. R. H. Michell, *Biochim. biophys. Acta* **415**, 81 (1975).
13. U. N. Wiesmann and N. N. Herschkowitz, *Pediatr. Res.* **8**, 865 (1974).
14. B. Disler, Thesis, University of Bern, Medical School (1983).
15. M. Stähelin and P. Simmons, *EMBO J.* **1**, 187 (1982).
16. H. Porzig, C. Becker and H. Reuter, *Naunyn-Schmiedeberg's Archs Pharmac.* **321**, 89 (1982).
17. J. E. A. McIntosh and R. P. McIntosh, *Mathematical Modelling and Computers in Endocrinology*. Springer, Berlin (1980).
18. B. B. Wolfe, T. K. Harden, J. R. Sporn and P. B. Molinoff, *J. Pharmac. exp. Ther.* **207**, 446 (1978).
19. M. D. Dibner and P. A. Insel, *J. biol. Chem.* **256**, 7343 (1981).
20. L. Hertz, S. Mukerij and J. S. Richardson, *Eur. J. Pharmac.* **72**, 267 (1981).