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Adrenergic Subsensitivity of a Cell-free Adenylate Cyclase from Rat Brain after Chronic Imipramine Treatment

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A particulate adenylate cyclase was prepared from cerebral cortex of rats which had been chronically treated with imipramine. Noradrenaline stimulated adenylate cyclase activity was significantly reduced compared to untreated controls. Neither total phosphodiesterase activity nor basal and maximal adenylate cyclase activity were altered by the imipramine treatment. This indicates the development of adrenoceptor subsensitivity which is functionally retained in the cell-free enzyme preparation.

Verminderte adrenerge Sensitivität einer zellfreien Adenylatcyclase aus Rattenhirn nach chronischer Imipramin-Behandlung.

Eine partikuläre Adenylatcyclase der Großhirnrinde von chronisch mit Imipramin behandelten Ratten wurde hergestellt. Es wurde gezeigt, daß die durch Noradrenalin stimulierte Adenylatcyclaseaktivität signifikant vermindert ist im Vergleich zu unbehandelten Kontrolltieren. Weder Phosphodiesterase noch basale oder maximale Adenylatcyclaseaktivität werden durch chronische Imipramin-Behandlung verändert, was auf eine herabgesetzte noradrenerge Empfindlichkeit der Adenylatcyclase hinweist, die in der zellfreien Präparation funktionell erhalten bleibt.

For several years, tricyclic antidepressant drugs, e.g. imipramine, desipramine or amitriptyline, have achieved widespread clinical use in the treatment of depression. These drugs, which are secondary or tertiary amines, respectively, are potent inhibitors of noradrenaline and 5-hydroxytryptamine uptake into presynaptic nerve terminals and these actions have been related to their therapeutic action. However, there is a large discrepancy between the time-course of the biochemical and pharmacological events, which are elicited by antidepressants within minutes or hours and their clinical therapeutic action which requires 1 to 3 weeks of drug therapy to develop.

Recently, it has been reported by several groups that chronic treatment of rats with tricyclic antidepressants results in a reduced adrenergic sensitivity of several brain regions as measured by noradrenaline stimulated formation of cyclic AMP[1–3]. All these experiments have been carried out using brain slice preparations. The present study was, therefore, intended to examine whether the adrenergic subsensitivity may also be demonstrated in *cell-free* membrane preparations. Such a cell-free preparation would offer several distinct advantages for further studies on the mechanism of action of antidepressant

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drugs. While experiments with brain slices require considerable amounts of tissue, cell-free enzyme preparations can be prepared from very small pieces of brain tissue thus permitting investigations of the effect of antidepressants on single brain nuclei. In addition, phosphodiesterase activity can be controlled and kinetic measurements will be possible with cell-free preparations of adenylate cyclase.

The purified membrane fraction from rat cerebral cortex used in these experiments represented to a large extend a synaptosomal preparation as evidenced by electron microscopy (data not shown). First, experiments were conducted in order to see whether chronic imipramine treatment has an influence on phosphodiesterase activity. As can be seen in table 1, neither the membrane bound nor the soluble phosphodiesterase activity was significantly altered by the prolonged drug treatment. This data substantiates the conclusion, that the observed adrenergic subsensitivity resides in the receptor-adenylate cyclase entity. Further experiments testing adenylate cyclase were carried out including 1 mM 3-isobutyl-1-methylxanthine as inhibitor of phosphodiesterase in the incubation mixture. Under these conditions phosphodiesterase activity is not any more detectable.

Table 1: *Phosphodiesterase activity in cell-free preparations of chronically imipramine and sham treated rats.*

	no treatment	chronic imipramine
	nmoles cyclic AMP hydrolyzed/mg protein/min	
Supernatant	11.3 ± 0.9	10.6 ± 0.9
Pellet	1.9 ± 0.2	1.6 ± 0.1

The activity of phosphodiesterase was measured in the particulate membrane preparation (membrane bound phosphodiesterase) and in the supernatant (soluble phosphodiesterase) of the first centrifugation step of the adenylate cyclase preparation (n = 4).

Noradrenaline, in a dose dependent manner stimulates the cell-free particulate adenylate cyclase (fig. 1), maximal stimulation being about 40 % above basal values (169 ± 6 and 168 ± 11 pmoles cyclic AMP/mg protein/min for untreated and imipramine treated rats, respectively). The shape of the dose response curve may be indicative of a biphasic stimulatory effect of noradrenaline, a first plateau being attained at 10 μM noradrenaline, while the maximal response is reached at 300 μM. Only at noradrenaline concentrations above 30 μM, however, can a significant subsensitivity be detected. The ED_{50} for noradrenaline is identical for both, drug treated and sham treated groups, irrespective whether the curve is regarded as monophasic (ED_{50} = 10 μM) or biphasic as drawn (ED_{50} = 60 μM for upper part, where subsensitivity is apparent). These findings can be correlated with data obtained by *Minneman* et al.[4] in receptor binding studies demonstrating that mainly β_1-receptors as opposed to β_2-receptors are effected by chronic desipramine treatment.

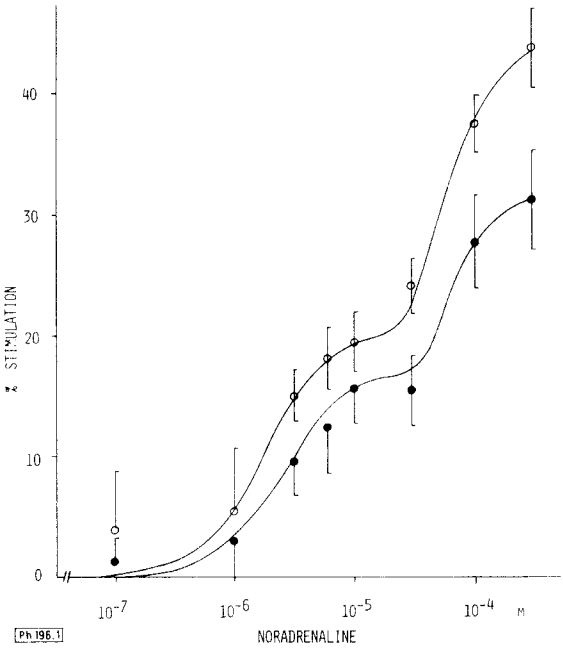

Fig. 1: Dose-response curve of noradrenaline stimulated cyclic AMP formation of a particulate membrane fraction from rat cerebral cortex. Open circles: control, filled circles: chronically imipramine treated rats (9 days). +: $2p < 0.05$.

That the reduced sensitivity of the adenylate cyclase to noradrenaline after chronic imipramine is related to a change in the receptor and not to a change of enzymic activity of adenylate cyclase, is shown by the fact that maximal activity of adenylate cyclase, as stimulated by 5'-guanylylimidodiphosphate (GMPPNP) and NaF is almost identical in brain membrane preparations from chronic imipramine treated and control rats (table 2).

Table 2: *Effect of GMPPNP, NaF and noradrenaline on cell-free adenylate cyclase from rat cerebral cortex.*

	no treatment	chronic imipramine
	% stimulation of cyclic AMP formation above basal	
GMPPNP (100 μM)	446 ± 28	482 ± 16
NaF (5mM)	536 ± 38	569 ± 45
Noradrenaline (100 μM)	38 ± 3	12 ± 1*)

*) $2p < 0.005$, n = 3, each experiment in triplicate.

So far, a direct action of imipramine or its metabolites, mainly desipramine, on the adrenoceptor could not be excluded. To answer this question, the levels of imipramine and desipramine in cerebral cortex after various periods of pretreatment were determined (table 3).

Table 3: *Imipramine and desipramine levels in cerebral cortex from rat after various periods of imipramine treatment.*

	Imipramine	Desipramine
	μ g/g wet weight tissue	
60 min after a single imipramine dose (50 mg/kg)	2.6 ± 0.3 (6)	2.2 ± 0.2 (6)
24 h after a single imipramine dose (50 mg/kg)	< 0.2 (6)	0.9 ± 0.03 (3)
5 d imipramine (30 mg/kg, 24 h after last dosage)	< 0.2 (4)	3.2 ± 0.07 (6)
9 d imipramine (30 mg/kg, 24 h after last dosage)	< 0.2 (5)	2.9 ± 0.4 (5)

Values are means ± S. E. M., number of experiments in brackets.

After 5 or 9 days of continuous imipramine administration the desipramine levels 24 h after the last dosage were in the order of 10 μM, disregarding any compartimentalization of the drug in brain tissue. The concentration of imipramine itself could not be quantitated at these time points because of the low amounts present (< 200 ng/g ww tissue). Thus, the action of imipramine is mainly attributable to its rapidly and almost quantitatively formed demethylated metabolite desipramine. Addition of desipramine at 10 μM concentration to the incubation mixture did not alter unstimulated and stimulated adenylate cyclase activity (table 4).

Table 4: *Effect of desipramine on cell-free adenylate cyclase from rat brain.*

	control	noradrenaline (100 μM)
	pmoles cyclic AMP formed/mg protein/min	
Additions		
None	218 ± 7	301 ± 15
Desipramine (2 μM)	239 ± 7	287 ± 15
Desipramine (10 μM)	208 ± 6	294 ± 15

Values are means ± S. E. M. from 3 experiments, each in triplicate.

Indeed, this had to be expected, since drug levels are rather high 60 min after a single imipramine treatment (table 3). At that time point, however, no adrenergic subsensitivity of the adenylate cyclase system was found in brain slices. In conclusion, the data presented here show that the postsynaptic adaptive changes in the sensitivity of the noradrenergic adenylate cyclase receptors described earlier in brain slices are preserved in a *cell-free* membrane preparation[1-3]. This will facilitate studies of the effect of chronic antidepressant treatment on anatomically small functional units of the brain. During chronic imipramine administration neither the total enzymic activity of adenylate cyclase nor soluble or particulate phosphodiesterase are altered to any significant extent. Also, the levels of antidepressants found after chronic application in the brain do not influence noradrenaline stimulated adenylate cyclase. These findings are, therefore, further support for the hypothesis that adrenoceptor subsensitivity develops as a consequence to the permanent inhibition of neuronal uptake of neurotransmitters by imipramine/desipramine. The phenomena, that the development of adrenergic receptor subsensitivity and clinical improvement of depression induced by chronic imipramine show similar time courses, may be coincidental or may indicate a cause-effect relationship. Further studies, in particular with antidepressant drugs of different chemical nature will be required to answer this question.

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Experimental

Male Sprague Dawley rats (140 – 160 g) were treated once daily with 30 mg/kg imipramine · HCl by gavage. Control animals received a corresponding vol. of water. The rats were kept at a 12 h light-dark cycle (7 – 19 h) with food and water ad lib.

Animals were killed by decapitation 10 days after commencement of drug treatment (24 h after the last imipramine application). The cerebral cortex was prepared as rapidly as possible (5 min) and homogenized briefly in 2 ml buffer (Tris · HCl, 48 mM, pH 7.4, $MgCl_2$, 12 mM, EGTA, 0.1 mM). After addition of 5 ml 1 mM – $KHCO_3$ the slurry was homogenized in a Dounce homogenizer (tight pestle, 30 strokes). The homogenate was centrifuged at 1 100 xg for 15 min. The pellet was dispersed in 10 ml 1 mM – $KHCO_3$ and centrifuged again at 12 000 xg for 20 min. This pellet was resuspended in 1 mM – $KHCO_3$ and the protein was adjusted to 7 mg/ml. Protein was determined according to *Lowry*[5].

The adenylate cyclase assay was performed according to a modified method from *Salomon* et al.[6]. The incubation contained in a final vol. of 280 µl 40 mM Tris · HCl, pH 7.4, 10 mM $MgCl_2$, 0.1 mM EGTA, 1 mM 3-isobutyl-1-methylxanthine, 14 mM kreatinphosphate, 70 EU kreatinkinase, 0.25 mM ATP including 0.5 µC α-^{32}P-ATP and 140 µg protein. The reaction was initiated by addition of ATP. The incubation (6 min, 37°) was stopped by adding 100 µl Tris · HCl buffer (190 mM, pH 7.6) containing 3.8 mM cyclic AMP including 50 nC ^{3}H-cyclic AMP for determination of recovery, 3.8 mM ATP, 1.9 % sodium dodecylsulphate). After dilution with 0.8 ml water the total solution was applied onto a Dowex WX 8, 100 – 200 mesh column. Purification of cyclic AMP was carried out exactly as described by *Salomon* et al.[6]. The cyclic AMP formed was quantitated by determination of ^{32}P radioactivity in a

liquid szintillation counter, and the results were corrected for yield as determined simultaneously by the added tritiated tracer. All data were transformed to pmoles cyclic AMP formed/mg protein/min. Phosphodiesterase was routinely assayed according to the procedure described by *Pöch*[7]. Imipramine and desipramine were determined according to described methods[8,9]. Recovery was monitored by internal standards of imipramine and desipramine.
Statistical analysis were performed using a two-tailed Student's t-test, all values are $\bar{x} \pm$ S. E. M.

References

1 A. Frazer, G. Pandey, J. Mendels, S. Neeleg, M. Kane, and M. E. Hess, Neuropharmacology *13*, 1131 (1974).
2 J. Schultz, Nature (London) *261*, 417 (1976).
3 J. Vetulani, R. J. Stawarz, J. V. Dingell, and F. Sulser; Naunyn-Schmiedeberg's Arch. Pharmacol. *293*, 109 (1976).
4 K. P. Minneman, M. D. Dibner, B. B. Wolfe, and P. B. Molinoff, Science *204*, 866 (1979).
5 O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. *193*, 265 (1951).
6 Y. Salomon, C. Londos, and M. Rodbell, Anal. Biochem. *58*, 541 (1974).
7 G. Pöch, Naunyn-Schmiedeberg's Arch. Pharmacol. *268*, 272 (1971).
8 U. Breyer, and G. Schmalzing, Drug Metab. Dispos. *5*, 97 (1978).
9 U. Breyer-Pfaff, T. Spribille, and I. Jahns, Biochem. Pharmacol. *27*, 1521 (1978).

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Warfarinanaloge 1,2-Oxathiolane

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Ein warfarinanaloges 1,2-Oxathiolanderivat vom Strukturtyp **4** wird dargestellt. Das Gleichgewicht zwischen den Ketoformen **11, 12**, der Enolform **10** und den cyclischen Halbketalformen **9** wird IR- und NMR-spektroskopisch sowie massenspektrometrisch untersucht. Bei einmaliger oraler Verabreichung an Ratten wird bis 250 mg/kg keine signifikante Verlängerung der Thromboplastinzeit (Quick) gemessen. Dies wird als weiterer[7] Hinweis gewertet, daß die Vitamin K-antagonistische Wirkung von 4-Hydroxycumarinen und analogen Verbindungen mit der Bildungstendenz der Enolform verknüpft ist.

Warfarin-Analogous 1,2-Oxathiolane Derivatives

3-[3-Oxo-1-(4-chlorophenyl)butyl]-5,5-dimethyl-1,2-oxathiolan-4-one 2,2-dioxide which is structurally related to warfarin has been prepared and investigated by i.r., n.m.r. and mass spectrometry.

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