1 Acknowledgments. We gratefully acknowledge the technical assistance of Joan R. Moor and Lakshmi Vulimiri with these studies, and the support of Grants No. 17249 and HL22410 from the U.S. Public Health Service.
2 To whom reprint requests should be addressed. Present address: Department of Chemistry, California State University, Fullerton, California 92634, USA.
3 Baker, G.T., and Schaefer, K.E., Effect of chronic hypercapnia on blood volume, plasma volume and red cell volume. Naval Submarine Med. Res. Lab. Report No. 604, 1969.
4 Cook, J.D., Hershko, C., and Finch, C.A., Storage iron kinetics. V. Iron exchange in the rat. Br. J. Haemat. *25* (1973) 691–705.
5 Cook, J.D., Hershko, H., and Finch, C.A., Storage iron kinetics. IV. The effect of inflammation on iron exchange in the rat. Br. J. Haemat. *28* (1974) 67–75.
6 Dacie, J.V., and Lewis, S., Practical hematology, 4th edn. Grune and Stratton. New York 1968.
7 Davidson, J., and Henry, J.B., eds. Clinical diagnosis through laboratory methods, 15th edn, p. 178. Todd and Stanford, 1974.
8 Faura, J., and Gurney, C.W., The effect of carbon dioxide on erythropoiesis. Ann. N.Y. Acad. Sci. *149* (1968) 456–461.
9 Giorgio, A.J., Cartwright, G.E., and Wintrobe, M.M., Determination of urinary copper by means of direct extraction with zinc dibenzyldithiocarbamate. Am. J. clin. Path. *41* (1964) 22–36.
10 Haskins, D., Stevens, Jr, A.R., Finch, S., and Finch, C.A., Iron metabolism. Iron stores in man as measured by phlebotomy. J. clin. Invest. *31* (1952) 543–557.
11 Hershko, C., Cook, J.D., and Finch, C.A., Storage iron kinetics. VI. The effect of inflammation on iron exchange in the rat. Br. J. Haemat. *28* (1974) 67–75.
12 Houchin, O.B., A rapid colorimetric method for quantitative determination of copper oxidase activity (ceruloplasmin). Clin. Chem. *4* (1958) 519–523.
13 Levine, W.G., and Peisach, J., Ethylene diamine tetraacetate, iron and ceruloplasmin activity. Biochim. biophys. Acta *77* (1963) 602–614.
14 Linder, M.C., Hixon, T., and Gonzalez, R., Distribution of copper among components of human serum in normal subjects and patients with cancer. Fedn Proc. Abstr. *42* (1983) 670.
15 Linder, M.C., Houle, P.A., Isaacs, E., Moor, J.R., and Scott, L.E., Copper regulation of ceruloplasmin in copper-deficient rats. Enzyme *24* (1979) 23–35.
16 Linder, M.C., and Munro, H.N., Assay of tissue ferritin. Analyt. Biochem. *48* (1973) 266–278.
17 Linder, M.C., and Munro, H.N., Iron and copper metabolism during development. Enzyme *15* (1973) 111–138.
18 Linder, M.C., and Munro, H.N., The mechanism of iron absorption and its regulation. Fedn Proc. *36* (1977) 2017–2023.
19 Linder-Horowitz, M., Ruettinger, R.T., and Munro, H.N., Iron induction of electrophoretically different ferritins in rat liver, heart and kidney. Biochim. biophys. Acta *200* (1970) 442–448.
20 Matrone, G., Interrelationships of iron and copper in the nutrition and metabolism of animals. Fedn Proc. *19* (1960) 659–665.
21 Munro, H.N., and Linder, M.C., Ferritin: structure, biosynthesis and role in iron metabolism. Physiol. Rev. *58* (1978) 317–396.
22 Noyes, W.D., Bothwell, T.H., and Finch, C.A., The role of the reticuloendothelial cell in iron metabolism. Br. J. Haemat. *6* (1960) 43–55.
23 Osaki, S., and Johnson, D.A., Mobilization of liver iron by ferroxidase (ceruloplasmin). J. biol. Chem. *241* (1969) 5053–5059.
24 Owen, Jr, C.A., Effects of iron on copper metabolism and copper on iron metabolism. Am. J. Physiol. *224* (1973) 514–518.
25 Ragan, H.A., Nacht, S., Lee, G.R., Bishop, C.R., and Cartwright, G.E., Effect of ceruloplasmin on plasma iron in copper deficient swine. Am. J. Physiol. *217* (1969) 1320–1323.
26 Ravin, H.A., An improved colorimetric enzymatic assay of ceruloplasmin. J. Lab. clin. Med. *58* (1961) 161–168.
27 Rice, E.W., Standardization of ceruloplasmin activity in terms of International Units. Oxidative formation of Bandrowski's base from p-phenylenediamine by ceruloplasmin. Analyt. Biochem. *3* (1962) 452–456.
28 Roeser, H.P., Nikles, A., and Cham, B.E., Effects of ascorbic acid deficiency on ferritin turnover; in: The biochemistry and physiology of iron, pp. 385–393. Eds P. Saltman and J. Hegenaur. Elsevier North-Holland, New York 1982.
29 Schaefer, K.E., Metabolic aspects of adaptation to carbon dioxide; in: Carbon dioxide and metabolic regulation, pp. 253–265. Eds H. Nahas and K.E. Schaefer. Springer Verlag, New York 1974.
30 Schaefer, K.E., and Baker, G.T., Effects of chronic hypercapnia on blood distribution in organs. Naval Submarine Med. Res. Lab. Report No. 603, 1969.
31 Schaefer, K.E., Messier, A.A., and Morgan, C.C., Displacement of oxygen dissociation curves and red cell cation exchange in chronic hypercapnia. Respir. Physiol. *10* (1970) 299–312.
32 Siimes, M.A., and Dallman, P.R., New kinetic role for serum ferritin in iron metabolism. Br. J. Haemat. *28* (1974) 7–18.
33 Van Wyk, C.P., Linder-Horowitz, M., and Munro, H.N., The effect of iron loading on non-heme iron compounds in different liver cell populations. J. biol. Chem. *246* (1970) 1025–1031.
34 Wixon, R.L., Prutkin, L., and Munro, H.N., Hemosiderin: Nature, formation and sign. Int. Rev. exp. Path. *22* (1980) 193–225.
35 Zähringer, J., Baliga, B.S., and Munro, H.N., Novel mechanism for translational control in regulation of ferritin synthesis by iron. Proc. natl Acad. Sci. USA *73* (1976) 857–861.

0014-4754/83/080850-06$1.50 + 0.20/0

Mini-Review

Molecular mechanisms in the action of imipramine

by E. Costa, D.M. Chuang, M.L. Barbaccia and O. Gandolfi

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeth Hospital, Washington, D.C. 20032 (USA)

For many years our understanding of how imipramine relieves some symptoms of depression and our theories on the etiology of affective disorders have progressed 'pari passu', almost as if pharmacology was leading the way. This association began when it was discovered that imipramine blocks the uptake of catecholamines and relieves the symptoms of depression. This observation suggested that imipramine

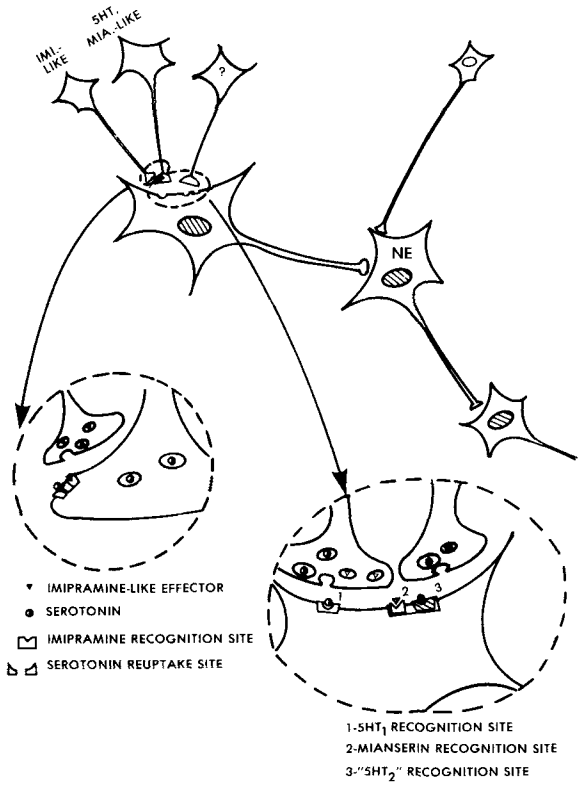
relieves the symptoms of depression because it inhibits norepinephrine (NE) uptake[1,2], thereby implying that a functional deficiency of adrenergic synapses is the cause of the symptoms[3]. When it was reported that chlorimipramine, which blocks serotonin (5HT) uptake with some selectivity also relieves the symptoms of depression, it was suggested that perhaps in certain forms of depression a deficit in serotonergic transmission[4] could be operative. Both theories lost appeal when it was considered that antidepressants block monoamine uptake almost instantaneously after their injection, but it takes 2-3 weeks of treatment before they ameliorate the symptoms of depression[1]. This realization gave relevance to the time constant as a factor to be considered in the correlation between pharmacological activity and mechanism of the therapeutic action of antidepressants. Since then, any new model to study the biochemical mechanism mediating the therapeutic action of antidepressants requires that the imipramine action to be considered appears after a latency of at least 10 days of treatment with daily doses of the drug.

It has been reported by various laboratories that in the brain of rats receiving repeated daily treatments with various typical antidepressants including imipramine, there is an attenuation of the increase in the activity of adenylate cyclase elicited by the addition to cortical slices or plasma membranes of isoproterenol or NE[5-9]. Furthermore, a long term treatment schedule with antidepressants can decrease the density of beta-adrenergic binding sites located in crude synaptic membranes prepared from brain[5-18]. This attenuation of beta-adrenergic receptor function appears after a time latency similar to that for the therapeutic action of various antidepressants therapies. A reduction in brain beta-adrenergic receptors function is elicited also by other therapies effective in the treatment of depression, such as the administration of MAO inhibitiors[12,14,18], electroconvulsive shock[6] and deprivation of rapid eye movement sleep[6].

The discovery by Langer and his collaborators that specific and high affinity binding sites for ^{3}H-imipramine are located in brain synaptic membranes[19-21] has provided a novel approach to study the molecular mechanisms that are operative in the imipramine action. These high affinity binding sites for ^{3}H-imipramine are recognized also by structurally related typical tricyclic antidepressants such as desipramine, amitryptiline, etc.[19-21], but are different from any of the high affinity recognition sites for known putative neurotransmitters[19,20]. When it was shown that imipramine binding sites are located on serotonergic axons[22,23] where they might be associated with the 5HT uptake mechanism[9,24], it became clear that the primary action of imipramine was on serotonergic transmission. The uptake of 5HT regulates receptor function by terminating the occupancy by the endogenous effector of the 5HT recognition sites located postsynaptically. This background information prompted studies to establish whether the imipramine modulation of the beta-adrenergic synapses required both the 5HT and NE axons. The necessity of intact NE axons was documented by showing that in rats with unilateral destruction of locus coeruleus[25] the down regulation of beta-adrenergic receptors caused by desipramine or iprindole was abolished unilaterally. The necessity of intact 5HT axons was documented by showing that in the cortex and hippocampus of rats with selective lesions of brain 5HT axons, there was no down regulation of beta-adrenergic receptor function following a long term treatment with desipramine[5] or imipramine[9]. The lesion of serotonergic axon terminals was elicited by a treatment with 5,7-dihydroxytryptamine given 40 min after an injection of desipramine or nisoxetine. The latter treatment was necessary to emphasize the selectivity of the lesion by inhibiting the toxin uptake by noradrenergic axons[26]. This information prompted studies of the molecular nature of the action of imipramine; obviously a great attention was centered on the site where imipramine binds with high affinity.

Several lines of evidence strongly suggest that ^{3}H-imipramine does not bind to the molecule that recognizes 5HT in the uptake process; perhaps, imipramine binds to a regulatory site that interacts with the 5HT carrier transport protein[9]. This view is supported by 1. the greater potency of imipramine in displacing ^{3}H-imipramine from synaptic membrane binding sites when compared with its potency to displace bound ^{3}H-5HT[24]; 2. the dissociation between the number of 5HT uptake sites and B_{max} of imipramine binding in the platelets of alcoholic cirrhotic patients[27]; 3. the consistent association between the reduction in the number of imipramine binding sites elicited by daily injections of imipramine[9,16,28] or desipramine[9,28,29] and the increase in the V_{max} for 5HT uptake[9]. All these observations taken together suggest that imipramine might act and label an allosteric site where an endogenous effector may act to control the uptake of 5HT in a negative manner. This endogenous effector(s) may be released intermittently from 5HT nerve terminals or other axons, to inhibit the uptake of 5HT into 5HT terminals[9]. Identification and purification of this endogenous effector is now in progress in our laboratory. The question then arises as to the quality of the action on serotonergic synaptic function elicited by long term daily injections of imipramine. The data available seem to indicate that this action is biphasic. Immediately after the first injection of imipramine the 5HT uptake is inhibited and presumably the transmission in serotonergic synapses could be enhanced. After daily injections, the drug reduces the number of specific binding sites for ^{3}H-imipramine and presumably the sites that mediate the physiological action of

the endogenous modulator of 5HT uptake are also reduced. Since the number of modulatory sites is reduced the overall reuptake of 5HT could be facilitated because one might infer that when a number of 5HT uptake sites are deprived of the site where the endogenous inhibitory modulator acts, a given quantum of 5HT released from a terminal causes a reduced postsynaptic response because a greater amount of 5HT is promptly taken up into 5HT axons. It can be argued that this decrease in serotonergic transmission elicited by a long term treatment with imipramine is responsible for the attenuation of the beta-adrenergic synaptic function caused by long term treatment with this drug. The question then comes as to why a destruction of 5HT terminals fails to change the beta-adrenergic receptor function[5,30] whereas a reduction of serotonergic function elicited by imipramine attenuates beta-adrenergic transmission. At this time, we do not have all the elements to explain this apparent discrepancy but we can postulate a working model to study this process (fig.). This model assumes that an interneuronal system links 5HT and NE neurons. Since it is currently believed that the serotonergic neurons function as pace makers[31], they may tonically sustain the intermittent firing rate of the interneuron which, in turn, excites noradrenergic cells. This inference imposes that without the serotonergic action, the interneuron will be inert, and NE axons will be modulated by other inputs.



The model of the figure takes into consideration the possibility that serotonergic axons produce 2 chemical signals and each signal acts on a different synaptic complex. One synapse is serotonergic, the other has 2 specific recognition sites, one for the signal produced by the 5HT axon and another to which bind ketanserin or spiroperidol, etc. The latter is called $5HT_2$ receptor and is modulated by the effector produced by 5HT axons which binds on mianserin binding site. This model is in keeping with the increase in the B_{max} of mianserin binding caused by a lesion of the serotonergic axons[22,32] and with the decrease in the B_{max} of $5HT_2$ binding site caused by several antidepressants[18]. Mianserin is another antidepressant which like imipramine down regulates beta-adrenergic receptor function[6] but it acts postsynaptically to 5HT axons[22]. Thus the supramolecular structure of 5HT synapses may include 2 units each one formed by a number of subunits. The 2 units are located in the membrane of the interneuron but only one of these units (fig., No. 1) is under the control of the 5HT uptake system which is affected by imipramine. The other (fig., No. 2) receives a chemical signal (cotransmitter?) from the 5HT axons which acts on the mianserin binding sites to modulate the $5HT_2$ recognition site (fig., No. 3). An excessive activation of receptor No. 2 of the figure, induced by long term imipramine through a release of the putative cotransmitter, causes a down regulation of $5HT_2$ recognition sites. Mianserin and perhaps iprindole down regulate $5HT_2$ because they mimic the cotransmitter which the model of the figure assumes to be released from the 5HT axons. One can infer that the pace maker function promoted by the serotonergic innervation is mediated by the cotransmitter produced by the serotonergic axons that acts on mianserin recognition sites (fig., No. 2). When the 5HT uptake is increased as a result of long term treatment with imipramine, the firing rate of 5HT axons may be increased to compensate the rapid regulation of 5HT. Hence the release of the endogenous agonist for the mianserin recognition site might be increased and thereby the pace maker function results accentuated. Accentuation of the pace maker function causes an excessive increase of sympathetic tone and an attenuation of beta-adrenergic receptor responsiveness. If there are no 5HT axons because they are destroyed there is no pace maker function and the NE receptor is not down regulated because the sympathetic tone is not increased[5]. However, even in the absence of 5HT axons the interneuron of the figure will still be activated by mianserin. The identification of the endogenous effector of the mianserin recognition site which may play a pivotal role in noradrenergic axon modulation, is currently being pursued in this laboratory.

Recently, Janowsky and coworkers[33] have confirmed that a lesion of brain serotonergic axon terminals with

5,7-dihydroxytryptamine prevents the down regulation of beta-adrenergic receptor binding sites elicited by desipramine. However, they reported that in rats receiving desipramine the down regulation of the isoproterenol sensitive adenylate cyclase could not be modified by the lesion. It is important to note that in Janowsky's report[33] the 5HT lesion increases the number of beta-adrenergic receptors. We fail to understand how an increase in the number of beta-adrenergic receptors is associated with a decrease in the receptor response to NE; such a dissociation was never reported to occur by other authors and goes against the present understanding of receptor function.

In conclusion the attenuation of cortical and hippocampal beta-adrenergic receptor function elicited by imipramine or desipramine requires the function of intact 5HT nerve endings[5]. Since a long term administration of imipramine or desipramine decreases the density of imipramine binding sites located on serotonergic axon-terminals and as a result enhances the uptake of 5HT into brain minces[9], one may surmise that a long term daily treatment with imipramine or desipramine decreases the amount of 5HT available at the synaptic cleft. Consequently, a smaller fraction of the 5HT released by the nerve impulse is available to activate the postsynaptic receptors, resulting in a decrease in the serotonergic transmission. The model of the figure implies that a decrease in serotonergic transmission triggers a compensatory increase in 5HT neuronal firing. Thus, 5HT and its cotransmitter are released at higher rates from serotonergic axons. The cotransmitter acts on mianserin binding sites and is the effector of the pace maker function that activates the interneuronal system that connects the 5HT with NE neurons (fig.). The release of this effector is obligatory for the down regulation of brain beta-adrenergic receptors elicited by imipramine, desipramine and other drugs acting on presynaptic imipramine binding sites. However mianserin mimics this activator and acts also without 5HT axons. According to the model of the figure, the function of the interneurons that modulate the interaction between NE and 5HT neurons leads to a balanced mood; depression may be associated with an impairment in the function of the interneurons which regulate the functional equilibrium between 5HT and NE systems. It is postulated that imipramine and desipramine relieve the symptoms of depression because they down regulate an increased responsiveness of beta-adrenergic receptors that might be associated with depression. This action depends on a modification of 5HT transmission mediated by their binding to the regulatory site of the 5HT uptake.

1 Klein, D.F., and Davis, J.M., Diagnosis and drug treatment of psychiatric disorders. Williams and Wilkins, Baltimore 1969.
2 Kuhn, R., Am. J. Psychiat. *115* (1958) 459.
3 Bunney, W.E., and Davis, J.M., Archs gen. Psychiat. *12* (1965) 568.
4 Van Praag, H.M., in: Handbook of psychopharmacology, p. 187. Eds L.L. Iversen, S.D. Iversen and S.H. Snyder. Plenum Press, New York 1978.
5 Brunello, N., Barbaccia, M.L., Chuang, D.M., and Costa, E., Neuropharmacology *21* (1982) 1145.
6 Sulser, F., in: Advances in biochemical psychopharmacology, vol. 31, p. 1. Eds E. Costa and G. Racagni. Raven Press, New York 1982.
7 Vetulani, J., and Sulser, F., Nature *257* (1975) 495.
8 Weiss, B., Heydorn, W., and Frazer, A., in: Advances in biochemical psychopharmacology, vol. 31, p. 37. Eds E. Costa and G. Racagni. Raven Press, New York 1982.
9 Barbaccia, M.L., Brunello, N., Chuang, D.M., and Costa, E., Neuropharmacology *22* (1983) 373.
10 Banerjee, S.P., Kung, L.S., Riggi, S.J., and Chanda, S.K., Nature *268* (1977) 455.
11 Sarai, K., Frazer, A., Brunswick, D., and Mendels, J., Biochem. Pharmac. *27* (1978) 2179.
12 Wolfe, B.B., Harden, T.K., Sporn, J.R., and Molinoff, P.B., J. Pharmac. exp. Ther. *207* (1978) 446.
13 Bergstrom, D.A., and Kellar, K.J., Nature *278* (1979) 464.
14 Sellinger-Barnette, M.M., Mendels, J., and Frazer, A., Neuropharmacology *19* (1980) 447.
15 Rosenblatt, J.E., Pert, C.B., Tallman, J.F., Pert, A., and Bunney, W.E., Brain Res. *160* (1979) 186.
16 Kinnier, W.J., Chuang, D.M., and Costa, E., Eur. J. Pharmac. *67* (1979) 289.
17 Clement-Jewery, S., Neuropharmacology *17* (1978) 779.
18 Peroutka, S.J., and Snyder, S.H., Science *210* (1980) 83.
19 Raisman, R., Briley, M., and Langer, S.Z., Eur. J. Pharmac. *54* (1979) 307.
20 Raisman, R., Briley, M., and Langer, S.Z., Nature *281* (1979) 148.
21 Briley, M.S., Raisman, R., and Langer, S.Z., Eur. J. Pharmac. *58* (1979) 347.
22 Brunello, N., Chuang, D.M., and Costa, E., Science *215* (1982) 1112.
23 Palkovits, M., Raisman, R., Briley, M., and Langer, S.Z., Brain Res. *210* (1981) 493.
24 Langer, S.Z., Moret, C., Raisman, R., Dubocovich, M.L., and Briley, M., Science *210* (1980) 1133.
25 Janowsky, A., Steranka, L.R., Gillespie, D.D., and Sulser, F., J. Neurochem. *39* (1982) 290.
26 Nelson, D.L., Herbet, A., Bourgoin, S., Glowinsky, J., and Hamon, M., Molec. Pharmac. *14* (1978) 983.
27 Ahtee, L., Briley, M., Raisman, R., Lebrec, D., and Langer, S.Z., Life Sci. *29* (1982) 2323.
28 Langer, S.Z., and Briley, M., T.I.N.S. (1981) 28.
29 Brunello, N., Chuang, D.M., and Costa, E., in: Advances in biochemical psychopharmacology, vol. 31, p. 179. Eds E. Costa and G. Racagni. Raven Press, New York 1982.
30 Mishra, R., Leiter, N.J., Steranka, L., and Sulser, F., Naunyn-Schmiedebergs Arch. Pharmak. *316* (1981) 218.
31 Aghajanian, G.K., in: Advances in biochemical psychopharmacology, vol. 34, p. 173. Eds B.T. Ho, J.C. Schoolar and E. Usdin. Raven Press, New York 1982.
32 Barbaccia, M.L., Gandolfi, O., Chuang, D.M., and Costa, E., Neuropharmacology *22* (1983) 123.
33 Janowsky, A., Okada, F., Manier, D.H., Applegate, C.D., Sulser, F., and Steranka, L.R., Science *218* (1982) 900.

0014-4754/83/080855-04$1.50 + 0.20/0