RESUME OF SCIENTIFIC CONTRIBUTIONS TO PSYCHOPHARMACOLOGY AND BIOLOGICAL PSYCHIATRY

While Fridolin Sulser was a visiting scientist from Switzerland in the Laboratory of Chemical Pharmacology at the National Institutes of Health in Bethesda, Maryland, he and B.B. Brodie utilized the reserpine-like syndrome as a "model depression" and in due course discovered the secondary amine desmethyl-imipramine (DM1) formed in vivo by oxidative N-demethylation of imipramine (1,2).DMI turned out to be the first selective inhibitor of the high affinity uptake of norepinephrine and triggered the development of many secondary amines of tricyclic antidepressants as therapeutic agents (e.g. desipramine, nortriptyline, protriptyline maprotiline, oxaprotiline, and more recently reboxetine). These drugs provided more selective pharmacologic tools to dissect the role of central noradrenergic mechanisms (3).

During the early 1970s, Fridolin Sulser and his associates discovered that treatment with antidepressants on a clinically relevant time basis (including tricyclics,

MAO inhibitors, some atypical antidepressants and electroconvulsive treatment) reduces selectively the responsiveness of the norepinephrine (NE) beta adrenoceptor coupled adenylate cyclase system in limbic and cortical structures of the rat brain (4,5). This deamplification of the central beta adrenoceptor system is often linked to a down-regulation of the B_{max} value of beta adrenoceptors, without changes in the KD. These findings shifted the research emphasis on the mode of action of antidepressant treatments and on the pathophysiology of affective disorders from acute presynaptic to delayed post-synaptic receptor - second messenger mediated events and emphasized the role of adaptive processes at the level of signal transduction and opened the gateway for subsequent studies on changes of programs of gene expression.

In 1983, Fridolin Sulser and his associates added the endocrine link to the aminergic system by demonstrating that glucocorticoids regulate the non beta component of the NE receptor coupled adenylate cyclase system (6). Thus, glucocorticoids represent in concert with catechol- and indoleamines, the third physiologically important group of regulators of the beta adrenoceptor-coupled adenylate cyclase system in brain. Since glucocorticoid receptors function as DNA binding proteins which can modify the transcription of specific genes, it became imperative to integrate the glucocorticoid receptor system into the amine hypothesis of affective disorders. In due course, the biochemical mechanisms of the regulation of this central beta adrenoceptor coupled adenylate cyclase system were unraveled (7,8,9) and the heuristic "serotonin/norepinephrine/glucocorticoid link hypothesis" of affective disorders was formulated, suggesting that the antidepressant sensitive, 5HT- linked and glucocorticoid responsive beta adrenoceptor system in brain functions as an

amplification/ adaptation system of vital physiological functions in brain e.g. mood, sleep, pain, neuroendocrine and central autonomic functions (10).

Results obtained by the use of non-linear regression analysis of agonist competition binding curves necessitated moving the "5HT/NE link" from the beta adrenoceptor level to sites beyond the receptors. Using C6 glioma cells in culture as a model system, the long expected convergence of receptor mediated neuronal and endocrine signals was demonstrated at the level of gene expression (11). These studies have provided the molecular basis for the "serotonin!norepinephrine/glucocorticoid link" hypothesis of affective disorders and the mode of action of antidepressants. They support the view that the antidepressant-sensitive, 51HIT-linked and glucocorticoid responsive beta adrenoceptor system may be involved in a much more general way as an amplification-adaptation system of stimulus transcription coupling and the regulation of brain specific gene expression. Results generated in human fibroblasts have demonstrated that the hypothesized convergence of signal transduction cascades at the level of protein kinase mediated phosphorylation of transcription factors is not hypothetical anymore. Using the nuclear transcription factor CREB as a target, both the activation of the cyclic AMP - PKA pathway by isoproterenol and the activation of the PKC pathway by the phorbolester PMA caused phosphorylation of nuclear CREB (12). This phosphorylation is additive in nature and occurs at the same molecular site (serine ^{133}). The results suggest that this convergence of neurotransmitter signals beyond the receptors at the level of protein kinase mediated phosphorylation may be a crucial mechanism of the mode of action of antidepressant drugs. Perhaps, when one individual neurotransmitter signal is relatively weak, the convergence of these signals at the level of protein kinase mediated phosphorylation may overcome such a

hypothetical weakness. The data provided a rational basis for the development of dual uptake inhibitors eg. venlafaxine and duloxetine.

Since the phosphorylation of CREB by PKA is mediated via the beta adrenoceptor - coupled adenylate cyclase, it was possible to test experimentally whether or not the antidepressant - induced deamplification of the beta adrenoceptor coupled adenylate cyclase was reflected beyond the second messenger system or whether the desensitization merely reflected a compensatory mechanism to offset the increased availability of synaptic NE (due to MAO inhibition or blockade of NE reuptake) with the overall rate of signal transduction being unchanged. Studies from our laboratory demonstrating that chronic but not acute treatment with noradrenergic antidepressants (DM1 and reboxetine) down - regulated the biologically active form of the transcription factor CREB (CREB-P) in the frontal cortex of rats (13) seem to support the notion of a net deamplification of the NE signal.The results are compatible with previous biochemical studies showing a reduction in the beta adrenoceptor cyclic AMP mediated formation of melatonin in the pineal gland (Heydorn et at. 1982.J.PharmacoLExp.Ther. 222, 534— 543) and a reduction in N-acetyltransf erase activity (Friedman et al. 1984. J. Pharmacol. Exp.Ther. 228,545-550) following chronic administration of various antidepressants despite a persistent increase in synaptic NE, caused by either MAO inhibition or blockade of Ne re-uptake.

While pursuing studies on the function of the "5HT/NE/ glucocorticoid link' system in brain as an amplification/adaptation system of signal-transcription coupling, Sulser and his collaborators provided the first provocative evidence that a tricyclic antidepressant (DM1) can regulate steady-state glucocorticoid mRNA levels in vivo by a mechanism which is independent of its effects on synaptic NE (14,15) thus providing a "proof of

principle" that blockade of biogenic amine uptake is not a prerequisite for antidepressants to exert profound effects in the CNS. This agonist- receptor cascade independent regu —lation of gene expression demonstrates the great potential to affect programs of gene expression by influencing targets beyond membrane receptors.

In collaboration with the Clinical Psychopharmacology Division, Fridolin Sulser and Richard C. Shelton have recently demonstrated an impairment of the activation of protein kinase A (PKA) via the beta adrenoceptor/cyclic AMP cascade in subcultured fibroblasts from patients with a DSM IV diagnosis of major depression (16,17). The blunted PKA response is associated with a reduction in the Bmax value of cyclic AMP binding to the regulatory subunit of PKA and is reflected in a decrease of nuclear CREB-P. Since phosphorylation processes are critically involved in both receptor adaptation and transcriptional activation, the elucidation of the underlying mechanism(s) and the study of the neurobiological consequences at the synaptic, cytoplasmic and nuclear level - using cDNA expression array techniques in concert with Differential Display Reverse Transcriptase - PCR methodology - became high priorities of the laboratory, promising to enhance our understanding of the molecular psychopathology of a major psychiatric illness. Importantly, the results obtained in human fibroblasts provided evidence that human fibroblasts represent a relevant model to study signal transduction - transcription coupling in patient with affective disorders (18). Among a number of apparently differentially expressed genes which we have amplified and cloned into the PCR - TRAP cloning system, we have paid special attention to a 269 bp sequence tag. This tag showed 95% identity with the H.

Sapiens PTX3 gene sequence in the 3' prime non-coding region. The densitometric analysis of slot-blots showed that the mean steady-state mRNA level of PTX3 was 3.5 fold higher in patients with a DSM-IV diagnosis of major depression as compared to that in normal controls (19). The results are of considerable interest because PTX3 is a member of the subfamily of long pentraxins potently induced by the cytokine IL-i beta which has been shown by Levine et al. (Neuropsychobiology 40:131 - 176, 1999) to be significantly elevated in the cerebrospinal fluid of hospitalized depressed patients. Recent results from our laboratory have shown the convergence of neuronal (via beta adrenoceptors), endocrine (glucocorticoids) and immune (IL ibeta) signals on PTX3 gene expression suggesting a potential role for this gene product in the integration of molecular and cellular activities of the nervous, the endocrine and the immune system. The results obtained in human fibroblasts catalyzed the overall concept of homeostasis and the view of dysregulation of molecular communication in and between the three integrative systems being the cause of or providing a predisposition to psychiatric illnesses such as depression.

In summary, the research activities of Fridolin Sulser have led to the discovery of the first clinically efficacious secondary amine of "noradrenergic" tricyctics and provided a rational basis for the development of new amine-selective antidepressant drugs and insight into their mode of action at the physiological, biochemical and molecular level. His scientific research, originating at the presynaptic neuron and progressing systematically to the receptor and to intracellular events beyond the receptor and finally to the nucleus, has generated many new heuristic concepts on the pharmacotherapy and etiology of depression (20,21). His scientific contributions to Psychopharmacology and Biological Psychiatry have been honored by many awards, among them the International

Anna Monika Award for" meritorious research in depression "(1937), the Gold Medal Award by the Society of Biological Psychiatry (1 986) for "significant and sustained work that advances an extends knowledge in Biological Psychiatry," a 10 year MERIT Award by the National Institute of Mental Health (1987), the election as an Honorary Fellow by the American College of Psychiatrists (1995) and the CINP Pioneer in Psychopharmacology Award (2006).

Representative Publications

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