DISCURSO DEL NUEVO DOCTOR HONORIS CAUSA D. KJELL FUXE

Rector, Deans, Padrino, Members of all faculties, colleagues, Ladies and Gentlemen

It is a genuine honour and pleasure to receive this doctor honoris causa from the University of Malaga with which I have strong ties since many years.

Fig.1

It all began in the 1960 ies when I was working as a medical student at the department of Histology, Karolinska Institutet. Professor Hillarp my teacher and friend had his large room upstairs behind this window and I was down here below somewhere.

Fig.2

Here is a photo of Nils-Åke Hillarp in1962.He tragically died in 1965 in malignant melanoma 1 month before my thesis on “Evidence for the existence of central monoamine neurons”

Fig.3

To-day I will mainly deal with the discovery made by Luigi Agnati and me and our teams of the existence of intramembrane receptor-receptor interactions and their molecular mechanisms, and functional and therapeutic implications.

Fig.4

This work began in 1979-80 by Luigi and me with radioligand binding experiments in brain membrane preparations.At the University of Malaga especially prof Jose Narváez and Dra. Zaida Diaz-Cabiale at the Department of Physiology made important contributions

Fig.5

But also Dr. Jose Aguirre at the same department contributed as well as Prof. Adelaida de la Calle and Dr. Alicia Rivera at the Dept. of Cell Biology and Dr. Inma Bellido at the Dept of Pharmacology and Clinical Therapeutics.

These excellent scientists have also previously been part of my Swedish team at the Karolinska Institutet for several years.

Fig.6

Over the years we have mainly worked with Dopamine and Noradrenaline transmitters in the brain which are the major members of the catecholamine transmitter family. The DA synthesis is illustrated in the fig as it starts with l-tyrosine and ends with dopamine formation via the work of the enzymes tyrosine hydroxylase and aromatic amino acid decarboxylase. Noradrenaline is formed by putting an hydroxyl group on the sidechain in the beta position.

Fig.7

This is from my thesis using the famous Falck-Hillarp technique to visualize the cellular localization of Dopamine (DA) and noradrenaline (NA) in the rat brain. By treatment with formaldehyde gas dopamine and noradrenaline are converted to fluorophors with a greenish fluorescence. In the fig the DA fluorescence in the nerve cells of the substantia nigra is shown in a well known paper together with Annica Dahlström from 1964.

Fig.8

We then went on to demonstrate in 1964 using this technique together with biochemical analysis made by Arvid Carlsson and Nils-Erik Anden and lesions by Knut Larsson that these DA cells gave rise to a crucial DA pathway densely innervating the striatum a major nucleus in the subcortical motor networks of the brain .Nobody believed us and it took about 10 years before it was finally accepted.

Fig.9

In 1964 -66 our team in Stockholm together with Anden and Larsson also mapped out the unique monosynaptic ascending and descending pathways of the noradrenaline and serotonin (5-hydroxytrytamine) neuron systems that globally innervated the brain and the spinal cord. With the Falck-Hillarp technique the serotonin is converted into a fluorophor with a yellowish fluorescence. In the scheme first made in 1965 the serotonin fibre pathways are also shown in yellow while the noradrenaline fibre pathways are shown in green .Again very few believed that such nerve cells could exist in the brain with nerve cell bodies in the lower brain stem forming vast terminal networks in all regions of the central nervous system (not shown) via monosynaptic pathways. It took again at least 10 years before their existence was accepted.

Fig.10

In this figure we illustrate that the central DA, NA and serotonin systems have a major clinical relevance. The nigrostriatal DA pathways when degenerating leads to Parkinson's disease. Overactivity in the meso-limbic DA systems is an important factor in causing schizophrenia and addiction. The locus coeruleus NA system globally innervating all the cortical areas of the brain is disturbed in stress disorders and in attention deficit hyperactiviy disorders. Finally, deficits in the raphe-limbic-cortical systems globally innervating the forebrain and diencephalon leads to depression.

Fig.11

In the 1970 ies our group moved over to the Berzelius lab that looks like a jail but it was there that Luigi and I discovered the first indications of the existence of intramembrane receptor-receptor interactions. It happened in 1979-80.

Fig.12

The discovery meant that receptors which are proteins in the surface membrane that bind and decode and transduce the transmitter signals like dopamine and noradrenaline into effects on the intracellular biochemical machinery and on the ion channels (changing the excitability and thus the firing rate of the nerve cells), are not separate but form supramolecular receptor complexes. These may be the true functional integrative units for decoding and transduction of signals reaching the cells.

Fig.13

In the past receptor-receptor interactions were only believed to take place via indirect mechanisms via changes in membrane potential or in phosphorylation that led to conformational changes in the other receptor as shown by Paul Greengard. Instead we introduced the hypothesis that direct receptor-receptor interactions existed, some times involving an adapter protein that mediated the receptor-receptor interaction.

Fig.14

So the field started with our first publications in 1980-81 and there was a dramatic rise in the interest in the receptor-receptor interaction field only when the first molecular evidence came around in 1998-99 with the discovery of the GABA B receptor heterodimer. The assembly of the same or different receptors were first discussed in 1982, a process called dimerization if only two or oligomerization if more than two receptors. The same year Luigi and I introduced the concept of receptor mosaics built up of clusters of multiple physically interacting receptors in the plasma (surface) membrane that could represent the molecular basis for learning and memory. Receptor mosaics are high-molecular-weight receptor complexes capable of “emergent properties”, i.e., of functions that could not be fully anticipated by analysing the characteristics of the single receptor (tessera) that form the receptor mosaic. A couple of years later we introduced the concept of circuit miniaturization that postulated computations to be carried out at the microscale level. Our first symposium on receptor-receptor interactions took place in Stockholm in 1985 but almost no one believed in our theory, mainly because the changes we discovered at the receptor recognition level were considered too small to be of biological relevance.

Fig.15

Here we illustrate how activation of one receptor in a heteromer can change the binding and signalling of the other receptor.

(i) Binding of the ligand induces a conformational change in the receptor.

(ii) Conformational changes are inter-molecularly transferred to the other receptor protein via their interfaces. Thus we are dealing with allosteric interactions, since the change occurs between two topographically distinct binding sites on the same protein.

A sequential propagation of the allosteric effects from one receptor to the following one may be possible and chains of interface allosteric interactions can contribute to the rapid and successful receptor mosaic assemblage and functions. This is the molecular basis for the receptor-receptor interactions.

Thus, the receptor-receptor interactions may cause cooperativity, since allosteric interactions are the basic mechanism for cooperativity. It should be noted that we can have negative or positive cooperativity in operation reducing or enhancing the binding and signalling of the other receptors in the receptor mosaic as illustrated in the figure.

Fig.16

Thus, to repeat in the past each receptor was on its own with the recognition-decoding process as a linearly organized process. Integration with other receptors took place in the cytoplasm and at the effector level.

Fig.17

With the new view the decoding process is a branched process already at the receptor level in the plasma membrane with receptor mosaic being the integrative unit with activation of multiple transduction systems and integrated activation of multiple effector systems and the generation of syndromic responses in the nerve cells.

Fig.18 (multiple)

This is to illustrate how the receptor mosaic concept was developed. Receptors are like tessera in arts like the Byzantian art in Ravenna, which are small cubes of different materials e.g. stones, marble etc.

However, the function of the receptor mosaic will depend not only on the receptor composition but also on their topography and their receptor interactions. Which will influence the emergent functions that arise.

Fig.19 (multiple)

Here different composition and topography of receptor mosaics are further illustrated. It is also shown that receptor mosaics interact with other proteins in the membrane to increase computation further and optimize RM function .There is the activation of various effectors via the classical phosphorylation. Cascades called vertical molecular networks controlling activation of transcription factors and thus gene expression as well as ion channel activity and thus the excitability and firing rate of the nerve cell.

Fig.20 (multiple)

Previously information handling was mainly believed to take place between cellular networks at the macroscale level. Based on the present work it becomes clear that information handling can take place also at the microscale level in molecular circuits in the plasma membrane where Receptor mosaics represent crucial nodes. It may be that in the human brain an increasing level of miniaturization through increases in molecular networks can help explain the increased capability of the human brain for learning and memory. However, most likely novel cellular networks may also have developed in the human brain which we have called mirror networks and make possible e.g. the formation of the internal theatre and of the universal grammer for languages. Thus an increase in the hierarchical level of integration has also developed in parallell.

Fig.21

I will now give you a few examples of receptor-receptor interactions which have strong clinical relevance. There exist dopamine receptor homodimers of the D2 and D1 subtype which mediate behavioural arousal and EEG activation. There also exist adenosine receptor homodimers of the A2A and A1 subtype which mediate sleep and behavioural inhibition. Adenosine is a modulator in energy metabolism formed from breakdown of ATP, adenosine triphosphate, that provides energy for the metabolic processes of the living cell. Adenosine via activation of its receptors aims to put the cell and the living organism to rest to allow the building up of new energy stores via formation of new ATP. Adenosine receptors bring this about in part by forming A1/D1 and A2A/D2 heterodimers where A1 and A2A receptors inhibit the D1 and D2 signaling, respectively. In this way behavioural arousal is reduced and sleep is favoured. It may be noted that caffeine in coffee gives arousal by blocking the A1 and

A2A receptors removing the constraint on D1 and D2 signaling in the above heteromers favouring wakefulness and motor activity.

Fig 22

A2A/D2 heteromers exist in a special motor system causing motor inhibition upon activation. In Parkinson's disease DA is reduced leading to reduced activation of the D2 receptors which therefore no longer can inhibit this motor inhibition pathway and remove this motor depression and strongly reduced motor activity develops. By removing the A2A brake on D2 signaling in the A2A(D2 heteromer by using A2A receptor antagonists a new strategy for treatment of Parkinson's disease has been developed based on the discovered A2A/D2 receptor interactions.

Fig.23

However, the A2A/D2 receptors may also form a receptor mosaic with metabotropic glutamate receptor 5 where this metabotropic receptor upon activation by glutamate a major transmitter in the brain synergize with the A2A receptor to antagonize D2 signaling, Thus adding metabotropic glutamate receptor 5 antagonists may give leveraged benefits of A2A antagonists in treatment of Parkinson's disease.

Fig.24

This aspect is further illustrated here where the standard therapeutic approaches based on acting only on DA receptors are illustrated. In Parkinson's disease high doses of l-dopa are given to replace DA and to overcome the antagonistic influence of A2A and mGluR5 on D2 signaling in this receptor mosaic.

The D2 receptor is the hub receptor i.e. the crucial receptor in this receptor mosaic controlling motor inhibition.

In another pathway the D2 receptor to the right again as a hub receptor controls emotional information from the limbic system to the cerebral cortex and enhanced activity at this receptor contributes to the development of schizophrenia. Therefore, antipsychotic drugs in high doses through blockade of these D2 receptors have been used to treat schizophrenia.

Fig.25

To-day novel therapeutic approaches can be developed based on receptor-receptor interactions using combined actions of drugs at the components of the receptor mosaic. Now low doses of l-dopa with less side effects can be used in **PD** by also adding A2A and mGluR5 antagonists, which removes the endogenous brake on the D2 signaling. In the case of **antipsychotic** treatment only low doses of D2 antagonists with less side effects may be used by combining the treatment with A2A and mGluR5 agonists which increases the brake on the D2 signaling

The future non-DAergic **antiparkinson and antischizophrenic** therapies may build substantially on the newly discovered existence of intramembrane receptor-receptor interactions in separate DA receptor-containing heteromeric receptor complexes in the striatum,including also a neuroprotective potential of such therapies.

Fig.26

Here you see the Retzius lab at the Karolinska Institutet where I am presently working

Fig.27 (multiple)

Here is a schematic representation of the “world” of the human being. The special features of the “positive pole” of the gradient underlines the special status of the human being. It may be interesting to note that for all the elements making up the “positive pole” a philosophy has been developed as indicated in the scheme (“utilitarianism”; “hedonism”; “aestheticism”; “categorical imperative”

Some philosophical problems and theories have a high impact on how to address scientific investigations. One of these issues is certainly the philosophical debate over innate ideas; which is central to the conflict between rationalist and empiricist epistemologies.

We started our reflections on the possible presence of innate ideas in the human brain from the observation that there exists strong experimental support for the view that not only complex behaviours (e.g., sexual courtship, parental care) but also aesthetic and ethic judgements may be, at least in part, genetically determined. On these grounds it is suggested that neurobiological findings can give important contributions to the philosophical debate on innatism by putting forward possible explanatory models and heuristic hypotheses

The uniqueness of the human brain in evolution may be the appearance of genetically determined special mirror networks in the cerebral cortex, probably of the nature derived network

(NDN) type, which code the logical principles to make abstractions possible. Examples of such types of networks are those coding the universal innate grammar for languages (Chomsky, 1965 and 1972). Of equal importance is the parallel development of genetically determined special mirror networks for emotions in the cerebral cortex that may encode ethical principles and aesthetics and are closely linked to the limbic system and to the analytical mirror networks. The special mirror networks may thus give the human dimension to our brains.

Recent studies support such a view since they indicate that specific neuronal networks are activated in aesthetic or in ethic judgements. Thus, it has for example been shown that left prefrontal dorsolateral cortex is selectively activated in humans during the perception of objects which they qualify as “beautiful”. With this follows also that the human being has special duties in life on our earth.

If you regard the human being in this picture as a scientist he or she is to-day faced with special problems due to the increasing complexities of the science and the increasing costs of the experiments making the negative pole strong. However, she or he can make a contribution if she or he can join a team of scientists with common interests and different expertise. Together they can make relevant contributions and if lucky develop e.g. novel treatments against diseases and reduce the suffering in the world.

Fig.28

Before Lisbeth and I came down to Malaga I saw our department through the branches and flowers of this magnificant tulip magnolia in colours of white and pink, and the department became part of another world that I felt much more at home in. But this moment filled with beauty was mixed with shadows by knowing that we are slowly ruining our Mother Earth given to us by God and sadness entered my soul.From within came the command. It is time to act; it is time to save our planet.

I am very happy and proud to receive this honour and have this genuine collaboration with my colleagues and friends at the University of Malaga and perhaps together we scientists may in a small way also help mother Earth stay alive the way she used to be giving a future also to our children and grandchildren and to the survival of mankind.