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REVIEW

Cell sensitivity, non-linearity and inverse effects

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It has been claimed that the homeopathic principle of 'similarity' (or 'similia') and the use of individualized remedies in extremely low doses conflicts with scientific laws, but this opinion can be disputed on the basis of recent scientific advances. Several mechanisms to explain the responsiveness of cells to ultra-low doses and the similarity as inversion of drug effects, have again been suggested in the framework of hormesis and modern paradoxical pharmacology. Low doses or high dilutions of a drug interact only with the enhanced sensitivities of regulatory systems, functioning as minute harmful stimuli to trigger specific compensatory healing reactions. Here we review hypotheses about homeopathic drug action at cellular and molecular levels, and present a new conceptual model of the principle of similarity based on allosteric drug action. While many common drugs act through orthostatic chemical interactions aimed at blocking undesired activities of enzymes or receptors, allosteric interactions are associated with dynamic conformational changes and functional transitions in target proteins, which enhance or inhibit specific cellular actions in normal or disease states. The concept of allostery and the way it controls physiological activities can be broadened to include diluted/dynamized compounds, and may constitute a working hypothesis for the study of molecular mechanisms underlying the inversion of drug effects. *Homeopathy* (2015) **104**, 139–160.

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Introduction

According to the 'similarity' (or 'similia') principle, patients can be cured using low doses or high dilutions/dynamizations of a drug that in healthy people produces similar symptoms to the disease. However, this approach is regarded as unscientific by some circles.[1,2] One current opinion about homeopathic treatment associates this medical approach with the placebo effect, either because of some highly publicized meta-analysis[3] or because of the alleged implausibility of its theories.[4–6] While controversies concerning the clinical efficacy have been recently discussed by others,[7] here we survey the homeopathic basic principles with the aim of examining their consistency with modern scientific knowledge of biological communication at cellular and molecular levels. The low dose effects and the healing power of pathogenic substances observed in homeopathy, are linked to the high sensitivity of biological systems at various levels of organization and to the multiform ways through which the complex of mind-body homeodynamic regulations ('vital force') reacts to external stimuli. Growing evidence from several science fields suggests that hormesis and paradoxical pharmacology are included in the 'adaptation' capacity of immune, neuroendocrine and cardiovascular systems, but also in cellular defence/repair mechanisms and even in molecular dynamics and conformational changes.[8–15]

Our working hypothesis is that recognition of the function of enzymes, receptors and signal transduction and their dynamic interaction with drugs, makes it possible to look at homeopathy in a new and fully rational light. The cornerstone of homeopathy – that the whole clinical picture of the individual patient to be taken into consideration – is not in dispute, but laboratory models also allow the

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Table 1 Glossary of the terms utilized

Subject/Term	*Definition*
Abscopal effect	A phenomenon sometimes observed in the treatment of tumours: The localized treatment causes not only a shrinking of the treated tumour but also a shrinking of tumours in different compartments.
Allosteric regulation	The regulation of a protein by binding a molecule at a site other than the protein's active site. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change. The term comes from the Greek allos (ἄλλος), 'other,' and stereos (στερεός), 'solid (object).'
Clathrates	The term, from the Latin 'clathrus' (= lattice or grating), denotes hollow formations in which the guest molecule is in a cage formed by the host molecule or by a lattice of host molecules. The cage is held together by very weak forces like hydrogen bonding, ion pairing, dipole–dipole interaction and van der Waals attraction.
Desensitization	Loss of responsiveness to the continuing or increasing dose of the same factor (agonist, antagonist, drug).
DNA microarray	Also commonly known as DNA chip or biochip, is a collection of microscopic DNA spots attached to a solid surface. Scientists use microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome.
Dynamization	In homeopathic pharmacopoeia, the dilution process is followed by vigorous shaking (see 'succussion') which is believed to provide the remedy with higher pharmacological power.
Energy landscape	A mapping of all possible conformations of a molecular entity, or the spatial positions of interacting molecules in a system, and their corresponding free energy levels. While a protein can theoretically exist in a nearly infinite number of conformations along its energy landscape, in reality proteins fold (or 'relax') into secondary and tertiary structures that possess the lowest possible free energy.
Epigenetic regulation	Functionally relevant changes to the genome that do not involve a change in the nucleotide sequence. Examples of mechanisms that produce such changes are DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying DNA sequence.
G protein-coupled receptors (GPCRs)	Large family of transmembrane receptors that sense molecules outside the cell (odours, pheromones, hormones, and neurotransmitters) and activate inside signals as cyclic-AMP and phosphatidylinositol. When a ligand binds to the GPCR it causes a conformational change, which activates an associated G protein by exchanging its bound GDP for a GTP. The protein chain passes through the cell membrane seven times.
Heat shock proteins	A group of proteins induced by heat shock, toxins or other stress (UV radiation, hypoxia). The most prominent members of this group are a class of functionally related proteins involved in the folding and unfolding of other proteins.
Hormesis	A phenomenon characterized by low dose stimulation, high dose inhibition of a biological system exposed to toxins and other stressors, resulting in an inverted U-shaped dose response curve.
Metallothioneins	A family of cysteine-rich proteins that have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals
Nanoparticle	A small particle composed by one or more compounds, including linked water, measuring 100 nm or less. Nanoparticulate matter has different properties from its bulk form in terms of mechanical, optical, electrical, magnetic, chemical, biological, and quantum behaviours. Nanoparticles cross membranes easily and act as highly reactive and catalytic agents.
Orthosteric site	Describing the primary, unmodulated binding site (on a protein) of a ligand. Orthosteric drugs usually block the active site of enzymes and receptors.
Paradoxical pharmacology	Term to refer to intriguing observations that chronic use of some drug types can have the opposite biological effect(s) to those seen following acute administration of the same drug.
Priming	A memory effect in which exposure to one stimulus influences a response to another stimulus. It may be homologous (same stimulus) or heterologous (two different stimuli).
State-space	In the theory of dynamical systems, is the set of values which a process can take. In physics, is an abstract space in which different 'positions' represent, not literal locations, but rather states of some physical system. When considering protein folding and free energy, this makes an energy landscape.

mechanism(s) of action of drugs to be investigated in animals, cells, tissues, and even at the molecular level. This 'reductionistic' perspective is not in contrast with the 'holistic' approach of homeopathy as a healing system, but rather represents the fundamental basis of a developing theory which would include recent advancements in complexity science and systems biology.[15–17] Given the holographic and fractal nature of all complex systems, the fundamental characteristics and rules of biological responses and related pathologies can be unravelled at any level of nature.

A glossary of the terms used is given in Table 1.

Cell sensitivity to ultra-low doses

The cell is the elementary particle of life and its sensitivity to external perturbations is the basis of both pathologic changes and therapeutic interventions. These changes at cellular level can be reduced to three dimensions: stimulation, inhibition, or differentiation. The higher the sensitivity to a regulatory factor – of a chemical, physical, or biological nature – the lower the dose or the energy capable of some 'effect'. The effects of homeopathic remedies in cellular models are well documented for a wide range of dilutions/dynamizations (Table 2).

When homeopathic drugs were tested in the same assay system at increasing dilutions, in most cases the greatest effects were observed at the lowest dilutions and also in Mother Tinctures (high molecular concentrations), but the same activity remained even at high dilutions.[20,29,30,43] In other cases, the effect was noted only in the low potencies.[31] In many instances, when the dilution exceeded the Avogadro constant, the effects appeared as pseudo-sinusoidal curves, with peaks of activity at certain dilutions/dynamizations, followed by inactive or less active dilutions.[19,29,53–56] Many of these effects have also been explained mechanistically as modifications of receptors, transduction mechanisms and gene expression changes. As there are many levels of cell regulation, there is no single mechanism explaining homeopathic effects, just as there is no single mechanism explaining the effects of conventional drugs.

High sensitivity to external regulations and nonlinear responses are frequently reported also with non-dynamized

dilutions. For example, recent studies measured the responses of mouse fibroblasts to TNF-α in terms of nuclear factor κB (NF-κB) gene expression.[57] A fraction of cells in the population can respond to concentrations of TNF-α as low as few pg/ml (10^{-12} g) and in a nonlinear manner. In fact, early NF-κB gene expression is not dependent on the concentration of the inducing signal. Moreover, the cell response was expressed as dynamical oscillations of NF-κB translocations, whose frequency can produce distinct gene expression profiles.[58]

It is worth pointing out that not all homeopathic effects can be reduced to the cellular level. A clear example is the recent finding that *Calcarea carbonica* induced regression of Ehrlich's ascites carcinoma in mice and 30−35% tumour cell apoptosis *in vivo*, but failed to induce any significant cell death when administered to cancer cells *in vitro*.[59] This apparent discrepancy was explained considering that *Calcarea carbonica* employs the immuno-modulatory circuit to assert its antitumour effects. Another example concerns carefully conducted experiments in which rats injected with prostate tumour cell lines developed fewer tumours when also exposed to the homeopathic remedies *Conium maculatum* 1000C, *Sabal serrulata* 200C, *Thuja occidentalis* 1000C, and *Carcinosinum* 1000C for 5 weeks.[60] However, the same drugs did not affect *in vitro* outcomes like cell viability and apoptosis gene expression, suggesting that *in vivo* effects are due to other mechanisms − possibly at a more systemic level − which are still to be clarified.

Effects on gene expression

Table 2 documents the ability of highly diluted compounds to modulate gene expression in human/animal cells and unicellular organisms. In experiments conducted in microbiological models (arsenite or UV treated *E. coli* and *Saccharomyces* cultures), ultradiluted *Arsenicum album* 30C or *Arnica montana* 30C modified the expression of specific genes that are the targets of arsenite and UV irradiation injury, respectively.[47–49] Ultrahigh diluted arsenic (45X treatment, containing a theoretical dose of As_2O_3 10^{-47} Mol/L) applied to wheat seedlings, poisoned with a sublethal dose of arsenic, produced a strong gene modulating effect compared to pure water (control).[46] Authors have suggested that diluted arsenic treatment induced a reequilibration of those genes that were up-regulated during poisoning. Mother tinctures and highly diluted drugs were tested in human tumour cell lines or cells challenged with carcinogens; they affected the modulation of the expression of specific mRNA markers (NF-κB, Akt, p53, Bax, Blc-2, caspase 3, Cyt-c) and increased tumour cell death.[22,41,50,51] These findings support the hypothesis that homeopathic remedies could turn some important genes on or off, initiating a cascade of gene actions to correct the gene expression that has gone wrong and produced the disorder or disease. In this hypothesis the relevant target gene should be sensitive to similar stimuli and exert a pleiotropic transcriptional regulation on a battery of genes with related functions. Needless to say, these *in vitro* effects are very interesting but cannot support the use of high dilutions in the treatment of cancer. In several studies cited above, not all the cells used, nor all the dilutions tested, gave positive results.

Preethi and coworkers investigated the effects of antitumour homeopathic medicines (*Ruta* 200C, *Carcinosinum* 200C, *Hydrastis* 200C, *Thuja* 1M and 200C) through the analysis of expression changes in the whole genome using DNA microarray.[23] *Thuya* 1M induced the expression change of a large number of genes (about 600), including genes involved in the process of apoptosis (up-regulated) and genes acting as second messengers in the process of tumorigenesis (down-regulated). The ultradiluted drugs also overexpressed diverse genes (about 100), which had a common role in induction of apoptosis.

Extremely low copper concentrations − from 10^{-6} Mol/L to 10^{-17} Mol/L − modified gene expression profiles of a human prostate epithelial cell line.[61] Many genes belonging to functional gene families (metallothioneins and heat shock proteins) were modulated by copper in a dose-dependent way, while others were modulated in a dose-independent way, at all concentrations tested.

Our results[30,31] prove the extremely high sensitivity of the human neurocyte gene network to centesimal dilutions (2C, 3C, 4C, 5C, 9 C and 30C) of *Gelsemium s.* As observed using DNA microarray analysis, *Gelsemium s.* 2C significantly modulated the expression of 56 genes (49 are down-regulated and 7 up-regulated) involved in neuronal functions (G-protein coupled receptor signalling pathways, calcium homeostasis, inflammatory response and neuropeptide receptors).

The expression of these genes decreased significantly, although with small changes, also after treatment with medium dilution (3C, 4C and 5C) and high dilutions of *Gelsemium s.* (9C and 30C). In the study conducted using the real-time PCR technique (RT-PCR Array),[31] exposure of a human neurocyte cell line to *Gelsemium s.* 2C dilution, containing a nanomolar concentration of the active principle gelsemine, induced a down-regulation of most genes of this array. In particular, the treated cells showed a statistically significant down-regulation of the prokineticin receptor 2, whose ligand is a neuropeptide involved in nociception, and in depression-like behaviour. In the latter study, the 9C dilution was not active.

The difference between the two gene-expression studies[30,31] was probably due to technical factors: while real-time PCR is the 'gold standard' for gene expression analysis of specific genes or small groups of genes, microarray is a powerful screening method for the whole genome and in our conditions exhibited higher sensitivity, detecting extremely low dose effects.

Further experiments conducted on resting epithelial cell lines treated with *Apis mellifica* MT, 3C, 5C and 7C dilutions[20] reported modified expression of hundreds of genes after 24 h incubation (MT: 391 genes up- and 495 down-regulated; 3C: 558 up- and 483 down-regulated; 5C: 132 up- and 168 down-regulated; 7C: 328 up- and 352 down-regulated). The main reduced functions were cytokine expression and inflammatory processes, while anti-oxidative responses and proteasome degradation were up-

Table 2 Effects of increasing dilutions/dynamizations (potencies) in cellular models *in vitro*, published in peer-reviewed literature

Potencies	*Test compound*	*Concentration of active principle*	*Cell type*	*Effect**
Mother tincture	*Phytolacca dec.*[18,19]	Not reported	Melanoma, breast carcinoma	↑ Cytotoxicity
Mother tincture	*Apis mell.*[20]	Not reported	Human prostate RWPE-1	↑↓ expression of different groups of genes (whole genome analysis)
Mother tincture	*Ruta grav.*[21]	16 mg/ml	Dalton's lymphoma ascites (DLA), Ehrlich ascites carcinoma	↑ Cytotoxicity
Mother tincture	*Carcinosinum*[22]	Not reported	DLA cells	↑ specific gene expression (p53 pro-apoptotic)
1M	*Thuja*[23]	10^{-3} of MT	DLA cells	↑↓Gene expression (whole genome analysis)
4X	*Podophyllum*[24]	10^{-7} Mol/L (podophyllotoxin)	Human neutrophils	↑ free radicals production ↓ Adhesion
4X–6X (complex)	*Arnica, Calendula, Hypericum* and *Symphytum*[25]	Not reported	3T3 fibroblasts	↑ Cell movement, chemotaxis
6X–12X (complex)	*Calcium fluor., Magnesium phos., Acidum silicicum*[26,27]	10^{-7} Mol/L (fluoride)	Rat osteoblasts	↑ Osteogenesis
2C	Histamine[28,29]	10^{-4} Mol/L	Human basophils	↓↓ CD203c expression
2C	*Gelsemium s.*[30,31]	10^{-9} Mol/L (gelsemine)	Human neurocytes SHSY5Y	↓↓ Gene expression (whole genome analysis, RT-array)
2C	*Gelsemium s.*[32]	Not reported	Cervical cancer HeLa	↑ Cytotoxicity
6X	*Phosphorus, Sulphur*[33]	10^{-6} Mol/L	Human neutrophils	↓ free radicals production
8X	*Magnesium phos., Manganum phos.*[33]	10^{-8} Mol/L	Human neutrophils	↓ free radicals production
3C, 5C, 7C	*Apis mell.*[20]	Not reported	Human prostate RWPE-1	↑↓ expression of different groups of genes (whole genome analysis)
5C	Gelsemine[34]	10^{-10} Mol/L	Rat neurons	↑ Neurosteroids
5C	*Lycopodium clav.*[35]	10^{-10} of MT	Cervical cancer HeLa	↑ Apoptosis
6C	*Mercurius sol.*[36]	10^{-16} Mol/L	Mice peritoneal macrophages	↑ Interferon-g production ↓ Free radicals production
6C	*Ruta grav.*[37]	10^{-12} of MT	Cell lines from brain cancer, leukaemia and melanoma	↑ Cytotoxicity in cancer cells (not in normal lymphocytes)
17X	Gibberellin[38]	5×10^{-18} Mol/L	Pea seeds	↑Germination
7X–24X (complex)	*Acon., Ars., Asa f., Calc. c., Chelid., Cinnamon, Conium, Echinacea, Gelsem., Ipecac., Phos., Rhus t., Thuya, Silicea, and Sulph.*[39]	Not reported	HT29 cells, human macrophages	↓NF-κB hyperactivity (reduced expression of reporter gene GFP in transfected HT29 cells), ↓TNF-α release in macrophages
9C	Gelsemine[34]	10^{-18} Mol/L	Rat neurons	↑ Neurosteroids
9C	*Gelsemium s.*[30]	10^{-23} Mol/L (gelsemine)	Human neurocytes SHSY5Y cell line	↓ Gene expression (whole genome analysis)
10C	Triiodothyronine[40]	10^{-24} Mol/L	Tail cells of Rana	↑ Apoptosis in cells treated with 100 nM T3
10C, 12C	*Nux vomica* or *Calendula*[41]	Not reported	Human gastric epithelial cell line KATO-III	↓ specific gene expression in *H. pylori* stimulated HB-EGF gene
15C	*Lycopodium clav.*[35]	10^{-30} of MT	Cervical cancer HeLa cell line	↑ Apoptosis
30X	*Rhus tox.*[42]	10^{-30} of MT	Primary cultured mouse chondrocytes	↑ specific gene expression (COX-2), ↑ inflammatory response (PGE2 release), ↓ specific gene expression (collagen II; de-differentiation role)

16C	*Histamine*[28,29,43]	*10^{-32} Mol/L*	*Human basophils*	*↓ CD203c expression*
14C–18C	*Adrenaline*[43]	*10^{-28}–10^{-36} Mol/L*	*Human basophils*	*↓ histamine release, ↓ CD 63 and CD203c expression*
15C–20C (pooled)	*Cadmium*[44]	*10^{-30}–10^{-40} Mol/L*	*Human lymphocytes*	*↑ Resistance to cadmium toxicity*
45X	*Arsenic*[45,46]	*10^{-45} Mol/L*	*Arsenic-intoxicated wheat seeds*	*↑ Germination*
				↓ Gene expression levels
30C	Arnica mont.[47]	*Not reported*	Escherichia coli	*↑ Resistance to RX toxicity*
30C	Arsenicum alb.[48]	*10^{-60} Mol/L*	Escherichia coli	*↑ Resistance to arsenicum toxicity*
30C	Carcinosinum[22]	*Not reported*	*DLA cells*	*↑ specific gene expression (p53 pro-apoptotic)*
30C	Arsenicum alb.[49]	*10^{-60} Mol/L*	Saccharomyces cerevisiae	*↑ Resistance to arsenicum toxicity*
				↓↑ expression of specific genes (apoptotic gene, stress response proteins)
30C	Thuja[50]	*Not reported*	*Mouse primary lung cells*	*↑ Resistance to benzopyrene toxicity, ↓ free radical production*
				↓ heat shock protein hsp-90 expression
30C	Condurango[51]	*Not reported*	*H460-non-small-cell lung cancer (NSCLC) cells*	*↓↑ expression of specific genes (apoptotic markers), ↑ Apoptosis, oxidative stress, mitochondrial depolarization*
100C	Sabal serr.[52]	*Not reported*	*PC-3 cancer cells*	*↓ Proliferation*
200C	Ruta grav.[21]	*Not reported*	*DLA cells*	*↑ Apoptosis*
200C	Mercurius sol.[36]	*10^{-404} Mol/L*	*Mice peritoneal macrophages*	*↓ Free radicals production*
200C	Carcinosinum[22]	*Not reported*	*DLA cells*	*↑ specific gene expression (p53 pro-apoptotic)*
200C	Carcinosinum, Hydrastis, Ruta or Thuja[23]	*Not reported*	*DLA cells*	*↑ Apoptosis, ↓↑ Gene expression (whole genome analysis)*

* All these results were reported as statistically significant. ↑=increase, ↓=decrease, ↑↓=different effects on different genes.

regulated. Groups of genes were diversely expressed by the MT or by 3C, 5C and 7C dilutions, suggesting a hormetic response. Other groups of genes were modulated only by high dilutions but not by the MT. On the basis of DNA microarray data, it has been suggested that gene regulatory networks may be regarded as dynamically 'critical' systems poised near the transition phase between order and chaos,[62,63] where extreme sensitivity to initial conditions and small perturbations are known to occur. Genetic regulatory networks may be the target of subtle messages by virtue of their flexibility in response to environmental stimuli.[63]

The physical–chemical state of the compounds employed plays a critical role. In fact, bio-active ingredients of medicinal plants (*e.g. Chelidonium*-chelidonine, *Lycopodium*-apigenin and coumarins) were nano-encapsulated in biodegradable poly(lactide-co-glycolide) (PLGA) polymers in order to increase their bioavailability and cellular uptake, and to boost their apoptotic effects on cells *in vitro*, compared with the corresponding free extracts; this hypothesis was supported by the results obtained.[64,65]

Also the crude extracts of *Phytolacca d.*, *Hydrastis c.*, *Gelsemium s.*, and *Thuya o.* were used to synthesize silver nanoparticles from aqueous solution of silver nitrate in ambient conditions; they showed the ability to interact with DNA, bringing about better stability, better drug bioavailability, and conformational changes in the DNA.[66,67] It is plausible that these preparations have greater effects on gene expression than free compounds, and the results presented by the authors support the hypothesis that those effects are due to efficient induction of apoptosis.

There is growing evidence for the presence of nanostructures in homeopathically-made medicines.[14,68–72] The role of nanoparticles presumably deriving from the containers and their interaction with the starting substance and the solvent during the dynamization process[32] may be critical since nanoscale forms are not identical in properties to bulk forms of a given material and nanoscale structure may lower dose requirements for triggering or regulating a given biologic event.[73–76]

Cell communication and regulation

In the body system, regulatory molecules bind to cell receptors which recognize chemical signals and respond to them with different actions. Following receptor stimulation, a panoply of transduction pathways are triggered, including G-proteins, phospholipid metabolites, protein kinases, calcium waves, proteolytic events, the formation of new complexes or dissociation of others, etc. The final outcome is the triggering or inhibition of effector mechanisms controlling various cell functions like gene expression, metabolism, secretion, movement, etc. All these steps may be the targets of possible pharmaceutical regulation.

Receptors

Cell receptors are proteins situated in the cell membrane or in the cytosol, like steroid receptors. They have different conformations and affinity with ligands, depending on the function they are conducting. Some of them act rapidly, others have a slower effect: this difference depends on the kind of receptor and how many steps there are in the process to evoke a response. There are several types of receptors, including membrane ligand-gated ion channels, G-protein-coupled receptors (GPCRs), kinase-linked and related receptors, and intracellular receptors controlling gene transcription.

According to the classic 'key-lock' model, receptors are specific and their activity is passive. However, a basal state of the receptors shows an activity without a ligand,[77] a discovery that changed current thinking about receptor functions and their regulation. In the absence of agonists, the level of basal receptor activity is determined by the equilibrium between the resting state and the active one. This equilibrium can be affected by the presence of specific agonists, inverse agonists, antagonists, and also by stochastic fluctuations or quantal effects based on individual responses.[78,79] An agonist may bind and stimulate the maximum activated state but, on the other hand, the inverse agonist shows affinity for the resting state and it acts by reducing the basal activity even more, thus producing biological effects opposite to those produced by an agonist.

Receptors for signal molecules or for other types of messengers (light, electromagnetic signals, stretching, action potentials) are highly plastic: the cells are capable of increasing (hypersensitivity, priming) or decreasing (desensitization, tolerance, adaptation, down-regulation) the number of receptors according to their needs, and also of regulating their activity by modifying the affinity for the signal molecule.

On occasion, the cells may present more than one receptor for the same molecule, but with different affinities and different intracellular effects. Intense stimulation at receptor levels may cause homologous desensitization but at the same time heterologous priming, *i.e.* increased response to other agonists (cross-sensitization). To give one example, high salt concentrations applied to the tongue desensitize receptors to salt but increase the sensitivity to low doses of capsaicin.[80] Changes of sensitivity are very important because they may involve the blockage of homeodynamics in chronic cases while at the same time prompting individual hypersensitivity to similar exogenous signals (drugs), i.e. acting on the same regulatory system. These new signals, in turn, may unblock the pathologic attractor and re-activate the homeodynamic networks, as previously outlined.[15,17,81–83]

Regulation and adaptation

Drugs induce an adaptation of the receptor and transduction mechanism that leads either to the loss or to a change in the efficacy of receptor autoregulation. Tolerance decreases the primary pharmacological effect and produces symptoms that are the opposite of the desired physiological situation. An example is GPCRs which stop activating second messenger cascades so that there is a decrease in the number of receptors expressed. This means that receptors are internalized inside the cells and/or that there is a decrease in receptor synthesis. The internalized receptors

can be re-cycled and used whenever the cell needs a greater response. For instance, if the ligand is not present in the required quantity, more receptors show up in the cell membrane, in order to capture as much ligand as possible; this reverse phenomenon is known as up-regulation.

Benzodiazepines (BDZs) are generally used in anxiety disorders when the organism suffers a spontaneous down-regulation of GABA(A) receptors, which means that the GABAergic circuit does not work properly. BDZs are GABA(A) receptor allosteric modulators, which enhance the effect of the GABA neurotransmitter that calms down the organism. One of the main problems in clinical use of GABA(A) receptor agonists is the development of tolerance and dependence. The anti-anxiety effect is significant if BDZs are used for a short period of time, otherwise they may cause rebound excitation due to their secondary action.[84] Most of these drugs, in long-term use and during withdrawal, have been associated with important modulations of receptor subunit expression in a brain-region-specific manner, participating in the mechanisms of tolerance and dependence.

Another example is morphine, an agonist of the Mu receptor; whenever there is an excessive presence of the ligand, the receptors are down-regulated by a balance mechanism inside the organism, and more pain is perceived.[85]

Hormones are both natural mediators and drugs which are subject to complex regulations and pleiotropic targets, including inverse effects according to the dose and the dynamics of delivery (*e.g.* continuous or pulsed-dose). A typical example of this situation is adrenocorticotropic hormone (ACTH), which causes the adrenal secretion of cortisol. This agent is also able to alter splenic lymphocyte activation; a paradoxical dose–response curve resulted when there are low or high level of ACTH. Very low doses (10^{-12}, 10^{-11} Mol/L) stimulated cell growth whereas higher levels (10^{-7}, 10^{-6} Mol/L) reversed the effect and are suppressive of lymphocyte growth.[86]

Pulsed-dose therapy provided by nasal administration of estradiol at the dose of 0.3 mg per day prevents bone loss in postmenopausal women[87] and reduces climacteric symptoms and is at least as effective as 2 mg oral estradiol or patch delivery, but with better tolerance.[88] In the latter trial, estradiol concentration reached a single peak of approximately 0.000014 mg/ml.

Signal transduction

GPCRs convey the majority of signal transductions across cell membranes. They are activated by diverse ligands, which vary from single photons (in rhodopsin) through ions, odorants, amino acids, fatty acids, neurotransmitters, peptides/polypeptides, to proteolytic enzymes, which cleave off receptor fragments to generate an activating ligand and adhesion molecules. One of the very special features of GPCRs is that they are highly druggable, to the extent that more than one third of all current therapeutic agents are directed at them.[89] On the other hand, they are also the target of bacterial toxins, and several oncogenic mutations are associated with these structures.

Intracellular systems couple receptor activation with production of signals or activation of effector mechanisms; they are associated with enzyme activities, variations of intracellular second messengers, modifications of membrane lipids and proteins, and the opening of ion channels. The level of responsiveness is controlled at various levels in the cell and is modified during the course of disease; differential expression and recruitment of these pathways provide a mechanism for subtle physiological regulation of cellular activity.[89]

Small changes in the concentration and/or oscillation frequency in second messengers like free calcium, protons (H+), cyclic adenosine monophosphate (AMP) and phospholipid derivatives can induce allosteric modifications of enzyme functions, chain-reactions and cascades of intracellular signals, and eventually gene expression. We showed in a leukocyte model that inverse effects of different doses of a bacterial agonist depend on the 'gating' exerted by cyclic AMP on the transduction pathways of different agonists.[90–93] Antigen-induced, IgE-mediated release of histamine from human basophils is an *in vitro* model of allergic reactions; it is blocked by extracellular histamine, presumably as a result of its ability to increase cyclic AMP levels.[94] Interestingly, this control is still preserved when histamine is highly diluted.[29,55]

Epigenetics

The epigenetic regulation of gene expression could be accounted for as an adaptive response to external stimuli due to the dynamic changes typical of this event. During the critical period of development, epigenetic alterations due to environmental stress permanently alter chromatin structure, thus affecting the function of cells and organs and increasing susceptibility to disease later in life.[95] For example in an adverse prenatal environment, like famine, a different pattern of methylation was observed for various genes involved in growth and metabolism,[96] associated with increased risk of obesity and cardiovascular disease in maturity.[97] However the effects associated with early-life epigenetic modulation could be also beneficial. For example, maternal dietary supplementation during pregnancy with methyl donors, like betaine, choline, folic acid and vitamin B12, is associated with reduction in the risk of developing neural defects, obesity, diabetes and cancer.[98] This adaptive epigenetic rearrangement can also serve to buffer the organism against environmental alterations.[99]

The finely tuned epigenetic modulation prompted by external detrimental events could be seen from a hormetic perspective. For example moderate stress, like modest physical activity and caloric restriction, has been shown to increase body fitness, prevent disease and improve quality of life through activation of antioxidant enzymes and proteins.[100] In yeast, transiently elevating mitochondrial oxidative stress, generally considered as pathogenic factors responsible for ageing, mediate a hormetic longevity signal[101]; this signal epigenetically modulates the chromatin binding capacity of the histone demethylase at subtelomeres, resulting in lifespan extension.

In some cases it has been shown that radiation to a localized area of the body can reduce tumour growth in a site outside that field of radiation.[102] This phenomenon ('abscopal effect') implies a complex interplay of different regulation pathways prompting a protective response from the whole organism.[103] The epi-reprogramming carried out by mildly-stressed bystander cells can prime distant cells for augmented defence against more threatening future insults.[104]

In this context, we would also mention the studies according to which epigenetic changes may have transgenerational transmission.[105] Exposure of a rodent to a non-lethal dose of common-use fungicide modifies the brain chemistry and behavioural response of the subsequent generations to stressors.[106] This is an important demonstration in an animal that ancestral exposure to an environmental compound modifies the susceptibility of descendants to a stress challenge experienced during their own life history.

In humans, a correlation of dietary intake of micronutrients in pregnant women and behaviour of offspring has been reported. For example, in a recent study an inverse correlation between maternal intake of iron and the risk of autism spectral disorders was found.[107] Early developmental periods are critical and prevention of iron deficiency might be a key for protecting against adverse neurodevelopmental outcomes. In context of epigenetics and the developmental origins of adult disease, it is noteworthy that exposure to dietary isoflavones during foetal development reduces the susceptibility to cardiovascular diseases and obesity in adulthood.[108]

Together, these findings suggest that an environmental event in one generation could affect the phenotype in subsequent generations, and these somewhat Lamarckian ideas are stimulating new interest in biology, ecology, and medicine.

Since evidence of gene expression changes induced by homeopathic drugs is accumulating, it is conceivable that some action is mediated by epigenetic modifications of related genes. However, direct proof of this possible molecular mechanism of drug action is still lacking. In our recent studies *Gelsemium s.* 2C treatment of SHSY5Y neurocytes reduced the expression of several genes[30,31] but did not induce marked general epigenetic changes. The only significant finding was an increased methylation (53% compared to 28% for the controls) of the homeobox A1 gene, which has a role in neural development and autism spectrum disorders (Olioso et al., unpublished results).

Sensitization and response to stress

Cells can respond to damage and stress by activating various repair and survival pathways. Low, sublethal doses of the same stresses provoke a prosurvival response, based on a number of molecular modifications.[109] These changes include Sirtuin-1, Histone deacetylase, AMP activated protein kinase, the Forkhead family of transcription factors, Nuclear factor-2 transcription factor, heat-shock proteins, DNA repair genes, antioxidant enzyme induction, receptor up-regulation, and proteasome activity. Also Bcl-2, an anti-apoptotic protein recognized by its antioxidant and prosurvival functions, has been shown to play an important role during oxidative-conditioning hormesis.[110] Suboptimal function of these mechanisms can lead to disease, while their stimulation by a 'small' heterologous stress may be one of the molecular mechanisms explaining post-conditioning hormesis at cell level.[12,111,112] A more general model for the action of homeopathic remedies based on stimulation of the organism's biological stress response network has been proposed by Bell and coworkers.[113] This model is based on the idea that the resilience and recovery from disease is due to time-dependent sensitization of host responses which reverse pathology direction.

In modern terms, the concept of 'susceptibility' due to both genetic and environmental factors can be compared with the original concept of 'miasm', as an idea of chronic disorders which make humans vulnerable to diseases. Although the concept of miasm is questionable by scientific pathology,[114,115] Hahnemann seemed to equate miasms to the different ways in which humans can be susceptible to various diseases; as scabies and venereal diseases were widespread in the 19th century, considering them − and their suppression with inappropriate medication of that time − as the source of vulnerabilities for other maladies is obvious.[116] Hahnemann also pioneered the concept of disease prevention when he stated that continuous exposure to noxious environmental influences undermines health (Organon, par. 77). In the same paragraph it is explicit that if a person can avoid noxious influences, he/she would lead a healthier life. The previous paragraph also mentions 'constant state of worry' as a noxious influence, showing Hahnemann as a forerunner also in the recognition of stress as a predisposing factor to illness.

The inversion of effects

Inverse and paradoxical effects of drugs constitute outcomes which are the opposite of those that would be expected from the drug's known actions. In medical history, Hahnemann was the first to consider a primary and a secondary action of medicines, the latter being the opposite of the former. In the Organon (par. 63−67), he mentioned three examples, the actions of wine, coffee and opium: "*The man who yesterday was warmed by too much wine (primary action) today feels chilly from every little draft (counteraction of the organism, secondary action). […] Excessive liveliness results from taking strong coffee (primary action), but afterward lethargy and drowsiness remain for a long time (counteraction, secondary action) unless removed by the repeated taking of more coffee (brief palliations). The heavy, stuporous sleep of opium (primary action) is followed on the next night by greater insomnia (counteraction, secondary action). […] Thus, to the primary action of every substance that in large doses strongly alters the condition of a healthy body our vital force always produces in the secondary action the exactly opposite condition (when, as stated above, such a condition exists).*"[117]

Modern pharmacology has recognized how such reactions arise in a wide variety of drug classes. Some are common; others have been noted in single case reports.[118] There are many possible mechanisms explaining the inverse effects of drugs, one of which is hormesis, with beneficial or stimulatory effects at low doses and adverse or inhibitory effects at high doses. Hormesis was originally applied in toxicology and radiation toxicity, but its importance is also obvious in pharmacology, since most drugs are essentially xenobiotics. Enhanced antioxidant defences, microsomal detoxification, clearance, augmented DNA repair, synthesis of heat shock proteins, or other compensatory responses have been offered as explanations.[109] The hormesis framework has been extended to include the influence of pre- or post-exposure conditioning and the similarity principle at the cell level.[12,17,92,111,112,119,120]

In homeopathy, a number of highly toxic substances are used in low doses or high dilutions as therapeutic agents. Two major examples supported by scientific literature and basic research are phosphorus[33,121–126] and arsenic.[48,127–139] Interestingly, the paradoxical anticancer power of the carcinogen arsenic was noted also by non-homeopathic literature.[140] Research data strongly suggest that arsenic influences distinct signalling pathways involved in mediating proliferation or apoptosis, according to different doses and target systems.

But hormesis is insufficient to explain the paradoxical effects of non-toxic substances such as antibodies or hormones. Moreover, the hormetic paradigm is still inadequate to include opposite responses in healthy and sick patients, which is the foundation of homeopathic treatment. Inverse effects of non-toxic molecules can be the consequence of secondary adaptations of cell receptors, which normally occur in numerous daily situations, such as after physical exercise, drinking coffee or taking a BDZ.

Caffeine is a stimulant due to its competitive antagonism of adenosine receptors, preventing the adenosine from acting as an inhibitor of the central nervous system. When there is chronic consumption, adenosine receptors are up-regulated[141,142] and the effect presents as headache, fatigue and apathy. The differential effects of caffeine in low and high users was demonstrated using functional magnetic resonance imaging, and showed up-regulation of adenosine receptors in high users, resulting in differential contributions of the neural and vascular effects of adenosine in the two study populations.[143]

In the marathon run, physical activity is linked to an increase of catecholamine levels, which is also associated with stress. Catecholamines are captured by alpha and beta adrenoceptors in order to activate the sympathetic nervous system. In the chronic exposure to these endogenous ligands (noradrenaline and adrenaline) found in trained runners, the concentration of plasma catecholamines in blood rises but receptor levels in blood cells decrease. Therefore, the surplus of catecholamines produces a down-regulation of the adrenoceptors.[144] The consequence of down-regulation of adrenoceptors is linked to a specific behaviour: the decrease in noradrenergic activity, in addition to endorphine production, causes the pleasant sensation deriving from physical effort and reduces pathologic reactions to stress.

Paradoxical pharmacology

Chronic heart failure (CHF) is pathophysiologically characterized by adrenergic overactivity resulting from the failure of cardiac pump function, leading to decreased blood pressure and tissue perfusion. Impaired beta adrenergic receptor signalling and function is a hallmark underlying mechanism of CHF[145]; use of β-agonists was found to give no benefit and to increase mortality.[146] But the paradoxical employment of β blockers (BBs) substantially decreased mortality in comparison with placebo and beta agonists.[147] Long-term BBs therapy is associated with an increase in myocardial beta-receptor density, significant improvement in resting haemodynamic output, and improved contractile response to catecholamine stimulation.[148]

The benefit noted in the treatment of CHF begs the question whether a similar paradoxical approach can be constructed for asthma, where the current therapy is still based on beta-agonists.[149] The therapeutic power of inverse effects is also exploited in the treatment of diabetes insipidus, where thiazides have long provided paradoxical antidiuretic benefit.[150] The importance of this emerging field is highlighted by a commentary paper in Trends in Pharmacological Sciences entitled "*Paradoxical pharmacology: turning our pharmacological models upside down*".[151]

Some paradoxical and bidirectional adverse effects are common and well known, *e.g.* agitation during paediatric sedation with midazolam, dysrhythmias from antidysrhythmic drugs, hyperalgesia from opioids, bradycardia with low-dose atropine, early suicidality with antidepressants, pulmonary oedema with diuretics.[118]

The basis of paradoxical pharmacology was clearly expressed by Bond[147]: "*Can exacerbating a disease make use of the body's compensatory and redundant mechanisms to achieve a beneficial long-term response? Can we use drugs that, according to traditional views, would be considered to increase stress on the system in the short term, to actually treat and cure disease in the long term? Is it possible to exacerbate disease for a longer-term gain? (…) Where to begin? If acute versus chronic responses are often opposite in nature, and if the contraindications have been made based on the acute effects, there is a suggested list where basic research can begin to look for clues to investigate paradoxical pharmacology. It is the list under 'Contraindications' because the opposite of contraindicated is indicated. A short-term discomfort may produce longer-term benefit.*" Similar concepts were expressed by Yun et al[152]: "*The modern medical paradigm based on blocking overactive pathways or augmenting deficient pathways offers symptomatic benefit, but tolerance to therapy can develop and treatment cessation can produce rebound symptoms due to compensatory mechanisms. We propose a paradoxical strategy for treating chronic conditions based on harnessing compensatory mechanisms for therapeutic benefit. The therapeutic effect*

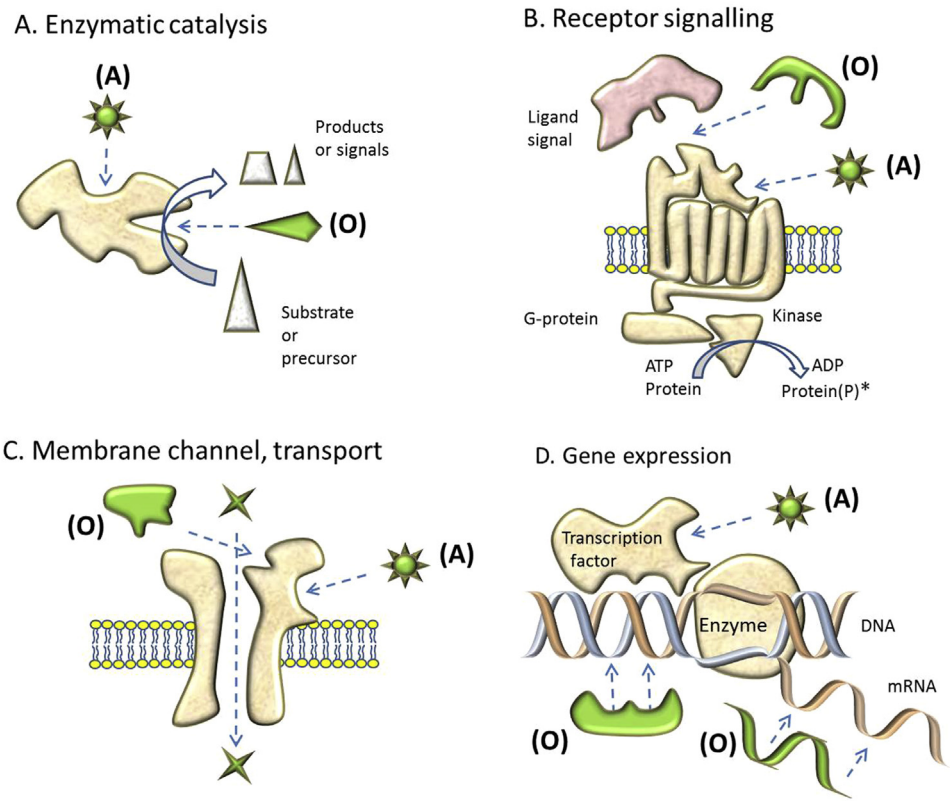

Figure 1 Allosteric and orthosteric drug actions. Allosteric (A) and orthosteric (O) regulation of different macromolecules. A: Enzymes; B: Cell receptors; D: Membrane channels and transport mechanisms; E: Gene expression at the promoter site.

is derived from compensatory response, rather than drug effect. (…) The concept may generalize to many other diseases, especially those involving pathways which exhibit strong homeostatic tendencies such as the neurologic, immune, and endocrine systems."

On the basis of these scientific facts, the logic of homeopathic reasoning is evident: if the body regulates itself in the opposite direction to the stimulus, we can use this property, giving low, sub-toxic, doses of pathogenic substances that trigger a counter-regulation. That this strategy is extremely close to the Hahnemannian concept of primary and secondary reactions to drugs has also been noted by others.[153] While the absorption and distribution of a drug (pharmacokinetics) is quite universal in a species, varying in a narrow range, the response to drugs (pharmakodynamics) is individualized, because it depends on multiple factors which, in turn, depend on the genetic and epigenetic characteristics of individuals, along with the current conditions at the time of treatment. At a particular dose (fixed or weight-based) in a population, a xenobiotic may produce anticipated effects with a range of effect magnitudes, including people who respond as expected, but also non-responders, excessive or toxic effects, and inverse effects. The proportion producing a paradoxical effect and the shape of the distribution differ for each xenobiotic and population.[118] Clearly, the homeopathic approach is individualized and no single drug can be used in all the persons affected by the same disease, a principle which is quite coherent with modern concepts of pharmacogenomics.

The Simile at molecular level: allosteric regulation

The specificity of any drug effect is based on the interaction of active principles with their biological targets (cells and molecules), and the same is conceivably true for homeopathic drugs.[15] At a molecular level, pharmacology recognizes the classic distinction between allosteric drugs and orthosteric drugs. Orthosteric drugs bind to the active site of a target enzyme or a receptor and block it; allosteric drugs bind elsewhere on the protein and indirectly alter the conformations at the active site (see Figure 1). Orthosteric drugs shut off native protein function; allosteric drugs modulate it.[154]

In the following sections we show how homeopathic drugs may work by exploiting the characteristic features of allosteric regulation. The fundamental issues of homeopathic drug action, sensitivity, specificity, nonlinearity and opposite effects depending on the context, are all rooted in the very behaviour of proteins and nucleic acids, the most important and informative molecular moieties of life.

Basic concepts of allostery

Allostery is a universal phenomenon in the chemistry of life, which in traditional terms may be regarded as the essential, tiniest, dynamic factor of the 'vital force'. The term comes from the Greek allos (ἄλλος), 'other', and stereos (στερεὸς), 'solid (object)', in reference to the fact that the regulatory site of an allosteric protein

is physically distinct from its active site. In fact, allosteric regulation is exerted by a factor acting at a protein's allosteric site, that is a site other than the active site where the function or the reaction mediated by the protein takes place. Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors.[155]

Long-range allostery is especially important in cell signalling and also in disease, where all co-occurring allosteric events (including posttranslational modifications, pathogen binding, and mutations) collectively tag the protein functional sites and regulate events like cell activation, proliferation and/or death.[156] Allosteric perturbation is common in cell physiology: it arises from noncovalent events, such as binding of ions, lipids, cyclicAMP, drugs, proteins, RNA, or DNA; from light absorption and from covalent events, such as phosphorylation, point mutations, or reaction with a small molecule. Allostery takes place in all dynamic protein single chains, in multimolecular assemblies and in RNA and DNA polymers.[157]

Figure 1 shows four examples of allosteric and orthosteric drug regulation of important biochemical and signalling cell functions, namely enzymes, receptors, ionic channels, and gene expression. All the current models hold that binding at the allosteric site regulates the activity (increasing or decreasing it) without blocking it, while orthosteric binding at the active site blocks the function of the protein in question. For example, the enzyme cyclooxygenase-2 is orthosterically blocked by aspirin but is allosterically regulated by non substrate fatty acids.

The activity of membrane-bound enzymes is controlled by the lipidic milieu in which they are embedded, as in the case of free-radical generation by nicotine adenine dinucleotide phosphate (NADPH) oxidase.[158] Interestingly, transition metal ions such as copper and zinc, which are crucial in many physiological and pathophysiological pathways,[159] also act as versatile allosteric modulators at low doses.[160] Allosteric signals transmit through multiple, pre-existing pathways; which pathways dominate depends on protein topologies, specific binding events, covalent modifications, and cellular (environmental) conditions. Consequently the observed functional, conformational, and dynamic effects will be different.[161]

The same principle also operates in pharmacology. Allosteric drugs present several key advantages over orthosteric drugs that target a protein's functional site.[157] They are highly specific because they do not bind in active sites, which tend to be highly conserved in protein families, and allow modulation of the protein activity rather than completely blocking it. Moreover, allosteric signals may propagate either within a protein, or across several proteins, to enhance or inhibit specific interactions along a pathway and specific cellular functions.[162] Allosteric drugs can activate a target protein not only by directly binding, but by modulating the interaction with another (activating or inhibiting) factor, and thereby create a mechanism of tissue-specific fine-tuning.[163]

Remarkably, the control of protein function and DNA gene expression is exerted not only by endogenous or exogenous specific molecules but also by low frequency electromagnetic fields,[164] ion resonance phenomena,[165] and water clusters.[166–170] Water molecules do not just play a part in solvation, but also function as chaperone catalysts participating in the regulation of chemical changes in living cells. A 'water-regulated cycle' consisting of this type of intracellular network of weak noncovalent connections may be presumed to exist in living cells.[15,171]

The protein energy landscape

Proteins are dynamic and flexible polymeric molecules with many degrees of conformational freedom; their final shape and function at a given time is determined by internal energetic interactions and by environmental conditions.[172] This can be clarified by the basic physical fact that biomacromolecules consist of ensembles of conformations with a certain distribution, which can be described by their free-energy landscape.[154]

As shown in Figure 2, if we plot the different possible conformations of a protein along the horizontal axis and the free energy along the vertical axis, we obtain what can be described as an energy state–space plot, or energy landscape. This is a representation of all the possible states of a protein, where higher-energy states (far from equilibrium) are unstable and have the tendency to descend towards more stable states, where free energy is lower. This map encompasses the native conformation as well as any nonnative conformations, which may form during folding or interaction with other molecules.[157] In living beings, the optimal utilization of energy is the main aim of the 'vital force' and is accomplished by biologic information, at genetic, epigenetic and systemic network levels.

The free energy landscape theory has transformed the field of protein folding.[173] According to this perspective, in the low-dimensional representations, because of the induced entropic contribution to the full free energy, the whole plot of the attraction basin appears funnel-like.[154] Depending on structural constraints and external conditions (temperature, chemical concentrations, pH and so on), a protein may belong to different sub-spaces, which lead it to naturally settle on different conformations.

Proteins with their different possible conformations 'explore' the energy landscape by searching the sub-spaces to find a narrow energetic alley that contains the protein's most favourable configurations. These sub-spaces have to be considered as 'attractors' in the energy landscape and represent the spontaneous emergence of ordered states from a variety of possible states; in this position, single molecules or molecular complexes have greater stability because they are situated in a local minimum within their energy state-space.

In the most simple picture, the protein can be viewed as populating one of two states: active or inactive (see Figure 2B–C). The basins of the two states are separated by a surmountable barrier, which allows the conformations to switch between the states. The model holds that, in the

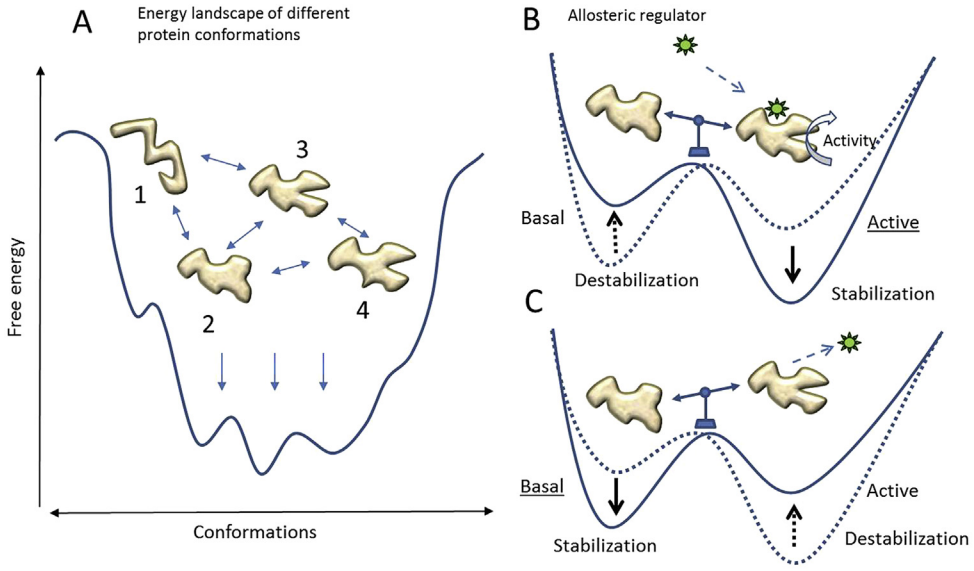

Figure 2 Graphic summary of how allostery works. Allostery is considered from the thermodynamic standpoint, in terms of the energy landscape of population shift. A: A simplified folding funnel of the state-space or energy landscape of a protein that occupies distinct energy minima; B: Allosteric two-state model presenting a balance between the inactive state (left) and the active state (right) binding to an allosteric ligand; C: detachment of the ligand destabilizes the protein energy and the population balance shifts to the left.

absence of any ligand (substrate or otherwise), equilibrium favours one of the conformational states. The presence of allosteric regulators 'perturbs' the free energy of the protein and changes its state-space landscape, stabilizing or de-stabilizing specific sub-spaces. In this way, the populations of active/inactive proteins distribute themselves in diverse attractors and the functional configuration of a given protein shifts to up- or down-regulated states. The population balance can be shifted to the active state (or vice-versa) through the noncovalent binding of one ligand or other minimal alterations at the allosteric site. When the protein is found near or at the top of an energetic barrier, its unstable position can be regarded as a bifurcation point of its trajectory in the space-state, where a minimal energy and informational influences — such as those exerted by highly diluted/dynamized compounds — could operate the 'switch' between active and inactive states.

Since allosteric sites are less conserved in evolution than orthosteric sites, they are subject to greater polymorphism in the population; consequently, the response to drugs becomes more flexible and individual. It depends on genetic predisposition (heredity), epigenetics (pathobiologic history, concomitant diseases, nutrition) and the context in which the drug is taken (receptor affinity, synergy, priming, desensitization).

Dynamics in health and disease

In Figure 3 the sequential changes of a model protein are represented in the space-states populated by the different conformations. The 'healthy' state of a protein — and of a cell — is normally characterized by a 'resting state' that minimizes energy loss and allows the protein to adapt and respond to perturbations (3A). Of course, if the protein is a functional repressor or inhibitor, its functional activity is normally 'ON', in the interest of the whole economy of the cell's energy expenditure.

Panel 3B shows the new state-space of the protein during interaction with an allosteric stimulator: if the protein is an enzyme, the result is a product; if we are considering a receptor, a specific signal transduction cascade is triggered, leading to some function. After completion of the required activity, the stimulus ceases, the stimulant is removed and the system spontaneously returns to the basal attractor (3C). This may occur through a variety of mechanisms such as inactivation of the ligand (*e.g.* acetylcholine in synaptic transmission), dilution and diffusion of the ligand (*e.g.* cytokines in inflammatory exudate), feed-back inhibition by the physiologic products of a reaction (*e.g.* most metabolic enzymes), or auto-phosphorylation of the receptor itself (*e.g.* epidermal-growth factor receptor). Despite the importance of this essential step, further analysis is outside the scope of this review.

The bottom panels of Figure 3 illustrate a conceptual model of the situation during pathology. Needless to say, diseases may have thousands of different causes and mechanisms and this is a schematic and oversimplified representation of one single, albeit essential, notion of general pathology. A perturbation of free energy is caused by a factor that binds to the protein, activating it in some anomalous, undesired way.

This pathologic factor may be exogenous (like bacterial toxins, pollution, viruses, radiation, etc.) or endogenous (like complement components, tumour-necrosis factor, oxygen free radicals, excess of hormones, etc.). The perturbation may be transitory or permanent, as in the case of genetic mutations. For example, aminoacid sequence mutations or binding of toxins can redistribute the ensemble to favour the ON state, making the protein constitutively active.[157] The resulting anomalous activity is translated to irregular functions and, directly or indirectly through a chain of further alterations, to disease symptoms and structural harm.

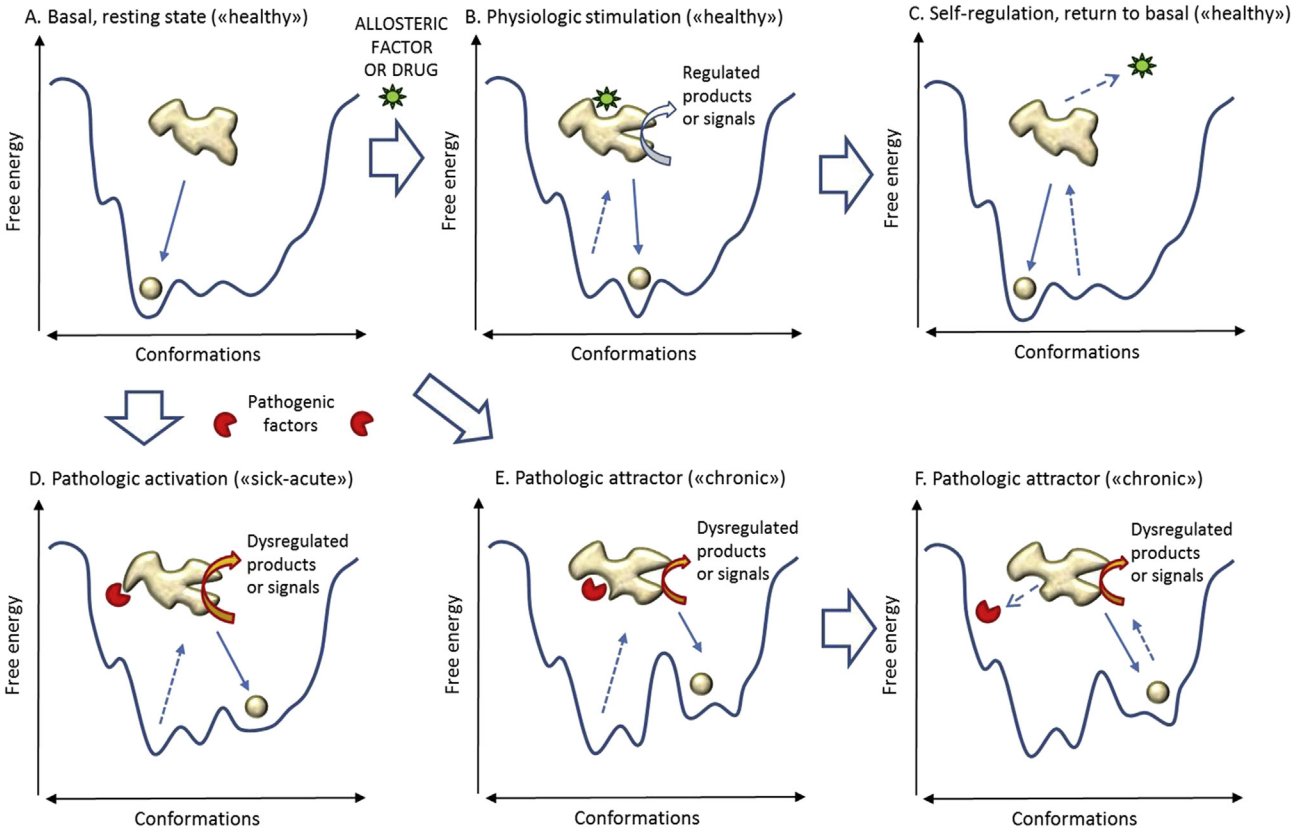

Figure 3 Changes of the protein energy landscape in normal (A–C) and pathologic (D–F) conditions. A: Resting state, where the basal activity and energy of the target protein system is low; B: The allosteric regulator enhances the activity in a normal, regulated way; C: Return to basal state after inactivation or detachment of the ligand; D: A pathogenic factor binds to the same protein and triggers a dysregulated activity, in a reversible way since the energy barrier is low and unsound (hallmark of acute diseases); E: The pathogenic factor markedly alters the structure of the protein in a way that is not spontaneously reversible (chronic disease); F: The protein is trapped in a minimum energy trough and can't return to normal shape even if the pathologic factor is no longer bound (self-maintained chronic disease).

Many oncogenic mutations of growth factor receptors or of protein kinases exemplify the latter case. Similarly, binding of virus proteins, neurotoxins, as well as the sun and UV irradiation, may all interfere with signalling *via* (often subtle) changes in the relative stabilities of conformational states in populations of proteins. For example, disruption of glucocorticoid steroid signalling plays a role in diverse disease states, including depression, leukaemia, and asthma.[157,174]

Schematically, pathologic modifications are represented by two different situations: acute (Panel D) and chronic (E and F). Acute variations are characterized by high energy consumption, marked symptoms, short duration and often by self-limitation. Spontaneous healing in acute cases is due both to the reaction itself, which is capable of eliminating (or killing) the pathogen, and to the small and easily removed energy barrier dividing the pathologic attractor from the normal one. Thus, when the pathogen is removed, the protein population exploring the space of states can easily recover the normal, physiologic, resting state.

In chronic pathology we have two possibilities, one in which the pathologic factor is strongly bound to the protein and is currently present (Figure 3, panel E); for example, in tuberculosis the mycobacteria are always present during disease, and the same is true for human immunodeficiency virus (HIV) infection, etc. In this category we can also include hereditary or acquired mutations of genetic information, which represent a permanent variation of the primary aminoacid sequence and therefore a strong constraint for the secondary structure and folding conformation.

In other instances, the pathologic factor gives rise to a disorder of protein dynamics that places the system in an attractor characterized by a stable self-organization separated from the healthy state (panel F). Therefore, even if the originating factor is no longer present, the system is unable by itself to find the way to re-enter the normal attractor basin. Examples are autoimmune and rheumatic diseases, originating from an infection and perpetuated by an internal disorder of the immune system which maintains the pathology even after elimination of the invading microorganism.

Allosteric dynamics and the 'Simile'

Recently, Nussinov et al.[154] addressed the question of how very similar ligands can bind at the same allosteric site, with one acting as an agonist and the other as antagonist. The chemical difference between allosteric agonists and antagonists – which bind at the same site – can be surprisingly small: even a change of a single atom or a small

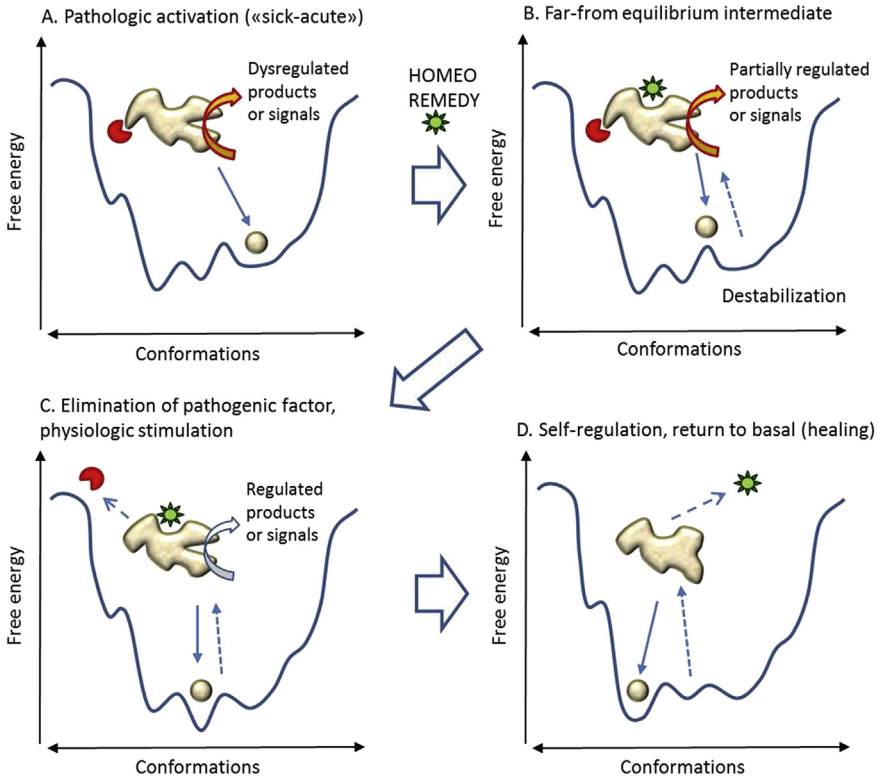

Figure 4 Protein energy landscape in pathologic conditions (acute case) and 'therapeutic' action of allosteric regulation. A: The protein system is pathologically activated; B: The allosteric factor destabilizes the protein and starts regulation; C: The pathologic factor is removed and the protein is normally activated; D: Spontaneous return to normal, healthy, state.

group may result in opposite outcomes or in substantial differences in efficacy. The slight structural changes between the conformations involve residues and hydrogen bonds that are responsible for the dynamic oscillations between states. A small change in the allosteric site increases the probability of a corresponding large change at the functional site, pushing the inactive conformation into the active state, or vice-versa. Further, even the same allosteric ligand bound at the same site can lead to opposite effects in different environments[175]: Tamoxifen has oestrogen antagonist activity in breast tissue but shows oestrogen-like activity in other tissues.

Figure 4 illustrates the hypothesis of allosteric action of the 'Simile' in acute diseases: the same substance that is capable of interaction with the allosteric site and of stimulating the protein in a normal situation is also capable of binding to the same protein in the pathologic state. The resulting composite shifts the configuration of the protein far from the natural pathologic attractor towards a new position (panel B), from which the situation of normal activation state is easily reached (panel C), partly because the reaction itself contributes to the elimination of the initial cause. Then, 'physiologic' stimulation is regulated as in a normal situation and this kind of stimulation is reversible to the resting, basal, state (Panel D).

In chronic disease (Figure 5), the dynamics of healing catalysed by the allosteric 'Simile' follow a sequence in which the drug first shifts the protein far from pathologic adaptation and lowers the energy barrier (panel B), after which the protein population gradually recovers a new configuration typical of physiologic activation (panel C), from which it spontaneously reaches the resting state when the influence of the allosteric drug ceases (panel D). In this case, too, the homeopathic principle lies in the similarity between the 'normal' configurations induced by the remedy in the healthy state (see Figure 3B) and the 'dysregulated' configurations typical of the unhealthy state (Figure 5A) which need to be treated. The remedy based on the similarity principle acts in the diseased state as an 'artificial' perturbation which is 'stronger' than the current pathologic configuration. The therapeutic action is stronger because its capacity to bind and affect its specific protein target has evolved by evolution as a natural way to recover the resting, energetically favourable, state.

There is some preliminary indication that homeopathic remedies may act through allosteric regulation of receptors. An example is *Gelsemium s.*, whose anxiolytic-like activity in rat neurons is inhibited by strychnine, a convulsant alkaloid, which acts as an allosteric inhibitor of the glycine receptor.[34,176] Homeopathic dilutions of *Ignatia a.*, whose major active principle is strychnine, have a small but statistically significant anxiolytic-like activity in laboratory mice.[177]

Symptom similarity at molecular level

A summary of the similia principle applied at the molecular level is shown in Figure 6. Since an active protein is linked to an active function, and the latter is expressed by 'symptoms', it is reasonable to conceive the 'simile' as

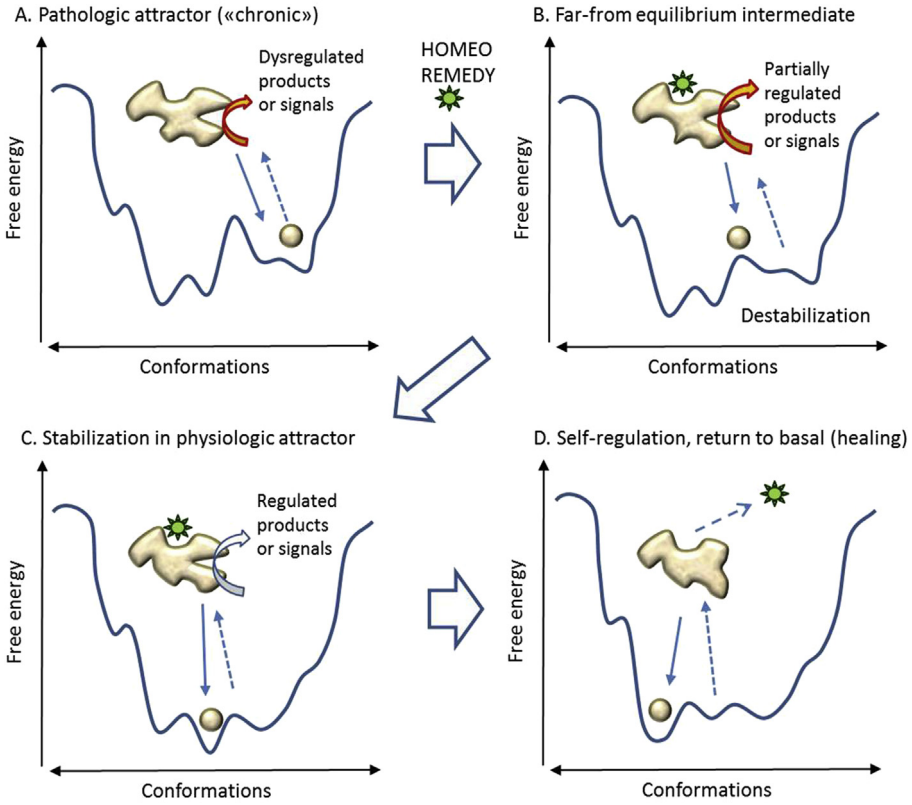

Figure 5 Protein energy landscape in chronic pathologic conditions and 'therapeutic' action of allosteric regulation. A: The protein system is chronically activated and dysregulated; B: The allosteric factor destabilizes the protein and reduces the energy barrier to a normal level (possible increase of activity); C: The pathologic conformation is no longer involved and the protein becomes activated in a normal way; D: Spontaneous return to normal, healthy, state.

an allosteric regulation of the same protein system in health ('Proving') and in disease (in this case represented by a pathologic attractor of the same protein). In connection with this illustration of the traditional 'Simile', we note that the protein target of allosteric regulation in pathology is the same as that considered in the healthy system above; therefore, since the activation of a protein produces specific 'symptoms' due to the perturbation of the related function, the 'symptoms' caused by this pathology (Figure 6, panel D) are 'similar' to those caused by activation in a normal state (Figure 6, panel B). The difference lies in the fact that during pathology those symptoms are manifested in a dysregulated way, for example they are too strong and painful, have longer duration and/or are present in anomalous sites in the body. Since in 'real' diseases a number of different proteins and functions are affected, and since all protein perturbations and consequent reactions are determined by the individual genetic constitution, each sick person presents a complex pattern of different symptoms.

This conceptual illustration of the 'simile' at molecular level is consistent with the traditional Hahnemannian view according to which drugs have two different effects: the primary and the secondary actions. The primary action is the actual effect of the drug on the healthy organism. The secondary action is the spontaneous and opposite action of the organism in the presence of an external substance, the drug, in order to restore a balance in the organism itself.

This is also connected with the use of very small doses of drugs in homeopathy. The effect of low and ultra-low doses is possible where the target systems, as a consequence of pathologic influences, are situated in far from equilibrium and unstable states in the energy landscape. It is tempting to speculate that the power of homeopathic treatment is the employment of high dilution medicines in order to lessen the primary action, the one similar to the disease, leaving active the secondary healing reaction. The latter effect depends not so much on the dose as on the sensitivity of the target system, which needs specific information about the complex pattern of various alterations of the whole person.

This view of the similarity principle confirms the theoretical advantage of homeopathy, embracing the principle of the secondary action of medicine to cure a disease. The homeopathic drug, acting on a sick organism in critically sensitive conditions, can move the system components — at molecular, cellular and systemic levels — away from energetically unfavourable pathological attractors, readdressing them toward more physiological ones.

Perspectives

Despite the preliminary and oversimplified nature of this model, the allosteric action of homeopathic drugs prompts interesting perspectives and working hypotheses:

a. Allostery works by preferential interaction of a signal with one state over the other (in simple terms, ON *versus* OFF), accompanied by a shift of the ensemble toward

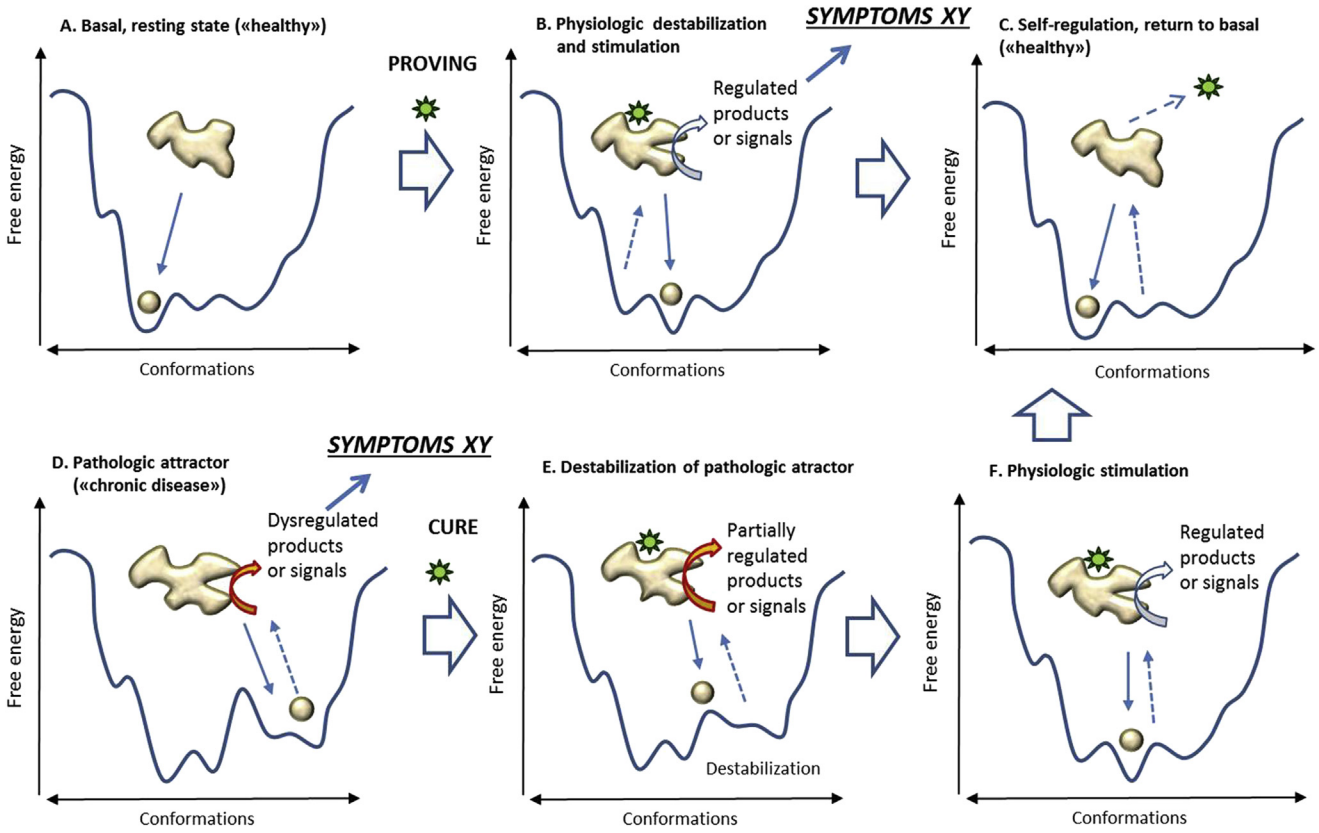

Figure 6 Summary of the similarity principle ('similia similibus curentur') based on allosteric stimulation of a functional protein system. A: Normal 'healthy' state; B: Stimulation and appearance of a pattern of symptoms, specific and typical of the molecular system(s) involved ('proving'); C: return to basal state; D: Chronic disease situation of the same molecular system(s), whose inappropriate activity produces a pattern of similar symptoms; E: Intervention of the 'Simile' signal which interacts with the same molecular system and starts normal regulation; F: Spontaneous self organization in the normal, physiologic attractor of the activated state, similar to 'B'; G: Spontaneous return to basal state ('healing').

this state (Figure 2B,C). Since the interaction signal-allosteric site is influenced by minimal differences in either the allosteric ligand or the receptor protein, this delicate conformational equilibrium may underpin pathology (*e.g.* excess basal activity of an enzyme) but also therapeutics based on the 'simile'. This scheme can be verified for homeopathic drugs by analysing their physicochemical state before and after dynamization. In fact, it is highly conceivable that dynamization, that involves high shearing forces, nanobubbles, oxygen free radicals, water clusters, etc.[178] may induce small but significant changes in the physicochemical nature of the active principles.

b. The effect of homeopathic allosteric intervention may also be accomplished by an indirect influence on the target protein system. For example, in the classic hormetic models, resistance and healing is due (also) to the synthesis of heat shock proteins, whose role is to refold the native proteins whose secondary structure has been damaged by toxic xenobiotics. In 'sick' cells, the synthesis of those proteins may be insufficient or suboptimal, and the low dose may act epigenetically on the promoter of the same or similar heat shock proteins, thus increasing resistance. This hypothesis is fully in keeping with the results of the long series of studies from the laboratory of Wiegant and Van Wijk.[12,179]

c. The scheme of Figures 5 and 6 also makes it possible to understand why using the 'simile' sometimes (but not always) may cause an apparent worsening of symptoms, when stimulation by the drug may be superimposed on the activity of the pathogenic factors. A change of attractor (in this case from pathologic to physiologic) requires the overcoming of an energy barrier, and this may require an increase of energy expenditure for a certain time, until the new attractor basin is reached.[180] However, the barrier dividing the two attractors may be very small and even small, subtle changes can make a difference.

d. In homeopathy, drugs are composed of natural substances which are never 'pure', *i.e.* they contain a number of different compounds. This means that allosteric regulations simultaneously have multiple targets, adding a noteworthy level of complexity and of pleiotropicity to the therapeutic intervention.

e. Signal transduction pathways and gene regulatory circuits underpin integrated cellular responses to perturbations, characterized by a number of mechanisms that amplify small percentage changes in the input signal into larger percentage changes in the output response. Ultrasensitivity is a form of nonlinear signal processing whereby a small fractional change in the input signal is amplified, producing a larger fractional change in the

output response.[181] Common types of ultrasensitive regulation in intracellular molecular networks are positive cooperative binding, protein multimerization, multistep signalling, positive feedback and stochastic resonance.[15] The importance for homeopathy of this kind of signal amplification — typical of biological networks characterized by nonlinear dynamics, adaptation and oscillation at the edge of chaos — has previously been suggested by us[180,182] and by others.[16]

f. The classic homeopathic method of drug discovery was originally based on careful observation of symptoms because at that time there was no knowledge of the 'intimate nature' of disease processes and of pharmacodynamic actions. The conceptual model presented here helps us to understand that the two perspectives — 'holistic', based on symptoms of the whole person and 'reductionistic', based on cellular and molecular changes — are not in contrast but have the same heuristic connotation. With the introduction of modern 'omics' techniques, the knowledge basis of homeopathic drug actions on healthy and sick people can be deepened to the molecular level.

These prospects, by increasing the credibility and plausibility of homeopathic concepts, allow us to include this pharmacological approach in the mainstream of modern science. However, plausibility is by no means certain proof, so research in this area still has a long way to go. It is important that homeopathic drugs, in different doses and formulations, be tested for pathways such as heat shock response, protein folding/unfolding, epigenetic mechanisms, gene expression and so on. Existing laboratory evidence of homeopathic drugs should be better linked with models of receptor function, cell regulation, and human findings (from both provings and clinical trials), in a system biology framework. How these concepts fit into homeopathy is important and requires further development.

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