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A redox cycling model for the action of β-adrenoceptor agonists[1,2]

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Summary. A cyclic redox mechanism for the action of β-adrenoceptor agonists is proposed. It has the following features: a) β-adrenoceptor agonists act by 'reductive activation' of the β-adrenoceptor (R); b) the redox state of R is reciprocally coupled to the redox state of the guanine nucleotide binding protein (G); c) binding of GTP to G reverses the agonist-induced alteration of the redox states of R and G; d) according to a specific version of the model the activation process involves a disulfide-thiol interchange reaction which leads to a GTP-revertible cross-linking of R and G by a disulfide bond. The way in which desensitization events may interfere with the proposed redox cycle is discussed.
Key words. β-Adrenoceptor; guanine nucleotide binding protein; redox cycle; disulfide-thiol interchange; desensitization.

Introduction

The catecholamines (adrenaline, noradrenaline, dopamine) represent an important class of neurotransmitters and hormones in the animal kingdom and in man. Their physiological and biochemical effects are quite diverse and often opposite (e.g. vasoconstriction or vasodilation, depolarization or hyperpolarization, stimulation or inhibition of adenylate cyclase). Furthermore, pharmacological and biochemical research has revealed the existence of at least six subtypes of catecholamine receptors: two α-adrenoceptors (α_1 and α_2)[9,18,71], two β-adreno-

ceptors (β_1 and β_2)[40] and two dopamine receptors (D_1 and D_2)[14]. In the light of this diversity of actions and receptors it is not obvious that a common mechanism of action of catecholamines can be assumed. Although cyclic AMP has been shown to be involved in many catecholamine-triggered processes as an intracellular signal or mediator, a role of cyclic AMP as 'second messenger'[61] has not been found in most α_1-adrenergic processes[18, 53] (see, however, Morgan et al.[42]) and has been repeatedly negated[17, 24, 38] in some β-adrenergic responses also. By contrast, guanine nucleotides, especially GTP, have been found to affect *all* catecholamine receptor systems, including the α_1-adrenoceptor system[7, 20, 37, 41, 56]. On the basis of abundant experimental evidence it is now generally agreed that guanine nucleotide binding proteins are mediator ('transducer') molecules which transmit the hormonal signal from the catecholamine receptors and a number of other hormone or drug receptors to another molecule, for instance to adenylate cyclase[50, 57]. In this paper, which attempts to rationalize a large body of experimental data on the effects of thiol- and disulfide-reactive agents in β-adrenoceptor systems[3, 6, 12, 16, 21, 23, 25, 26, 32, 36, 43, 44, 58, 60, 65–70, 72], the interaction of only two proteins is considered: the β-adrenoceptor (R) and the guanine nucleotide binding protein (G). The redox model described below bears some resemblance to a hypothesis put forward by Robillard and Konings[49] for the role of dithiol-disulfide interchange in membrane-related processes; actually, it may be regarded as an adaptation of the Robillard-Konings hypothesis to the peculiarities of β-adrenoceptor systems.

The redox model

The essential features of the model are as follows:
a) R is able to oscillate between a resting (oxidized) state and an activated (reduced) state; catecholamines or synthetic sympathomimetic agents act by 'reductive activation' of R. [Also noncatechol β-adrenoceptor agonists (e.g. dichloroisoproterenol, pindolol) which are usually classified as 'nonreducing' have a sufficiently high propensity to donate an electron to a suitable acceptor (data not shown).]
b) The redox state of G is reciprocally coupled to the redox state of R, i.e. when R is reduced, G is oxidized and vice versa. In other words, R and G form a redox couple according to equation (1):

$$R_{ox} + G_{red} \leftrightarrows R_{red} + G_{ox}. \quad (1)$$

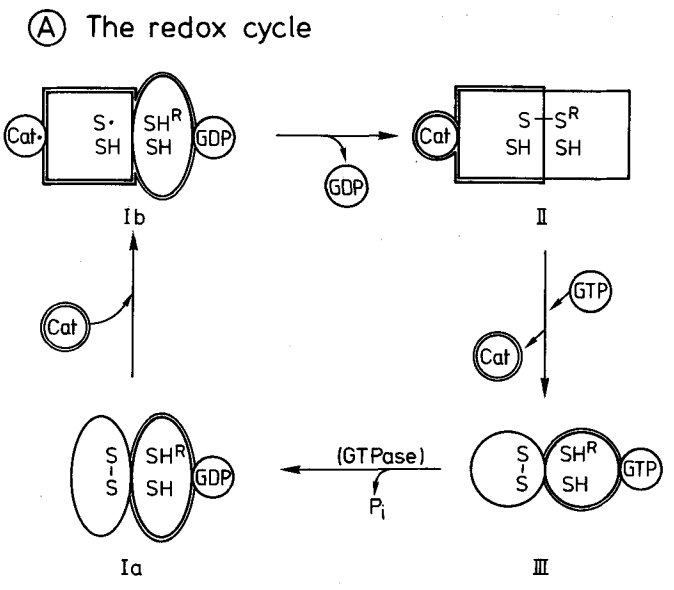
c) Binding of GTP to G reverses the catecholamine-induced alteration of the redox states of R and G, i.e. GTP causes re-reduction of G, which is coupled with reoxidation of R. In this way the initial (resting) state of the system is restored and a new cycle of catecholamine activation can be initiated.
d) In a specific formulation of the model, the simplest case of a cyclic redox process, namely a one-electron shuttle between R and G is proposed, as it takes place in the overall equation (2)

$$R\begin{vmatrix}S\\S\end{vmatrix} + {}^{SH}_{SH}G \overset{A}{\underset{GTP}{\rightleftarrows}} R^{S-S}_{SH}{}^{SH}G \quad (2)$$

where $R\begin{vmatrix}S\\S\end{vmatrix}$ denotes R containing an essential disulfide bond[12, 21, 36, 66, 72], A stands for agonist and ${}^{SH}_{SH}G$ denotes G, supposed to possess two critical and possibly vicinal thiol groups, one of which (subsequently designated SH^R) is able to react with an agonist-induced thiol group of R to form a disulfide bond which covalently cross-links R and G in a GTP-revertible manner.
Individual steps and some mechanistic details of the overall reaction (2) are described in the figure in which the proposed redox cycle is integrated into current concepts – somewhat differing from author to author[4, 33–35, 46, 50, 51, 57, 62] – on the interactions of agonist, R, G and guanine nucleotides. An essential feature of the model is a transient opening of a disulfide bond on R which is immediately followed by a conformational change of R, thus preventing the reformation of the original disulfide bond in favor of a new disulfide bond. This new disulfide bond cross-links R and G and causes a 'locking'[32] of the agonist on R as well as a conformational change of G. The locked state of the agonist should be short-lived in order not to block the system which should respond quickly to changing catecholamine concentrations. This is achieved by binding of GTP to a site which allows it to influence the SH^R-group, thereby raising the pK-value of the SH^R-group and favoring its reduced state[49, 64]. Consequently the disulfide bond between G and R is cleaved under reformation of the original disulfide bond on R. Rapid release of the agonist in the GTP-induced low affinity state of R and subsequent hydrolysis of the bound GTP into GDP and inorganic phosphate by a GTPase[11] completes the cycle. In the figure a radical mechanism for the fission of the disulfide bond is proposed which is consistent with the high reducing potential of catecholamines[19, 22, 39] and their ability to form semiquinone radicals[5, 28–30, 47, 73].
A common feature of the model presented here (fig.) and those of other investigators[4, 33–35, 46, 50, 51, 57, 62] is a cyclic change in the affinity of R for an agonist. The question of whether the high- or low- or any intermediate affinity form of R is the 'active' one does not appear very fruitful since, as pointed out in a review of Birnbaumer and Iyengar[4], both the high- and low-affinity states of R are sequentially required for the overall activation process. The minimal time needed for R to pass through the whole cycle is obviously dependent on the rate constants of the individual reactions which are assumed to occur in a strictly sequential manner. An important question is, which reaction is rate-limiting for the overall activation process under 'normal' physiological conditions or under conditions of sub- or supersensitivity to the agonist. A disulfide-thiol exchange reaction, wherever it may occur in the activation cycle, might play an important role in this respect.

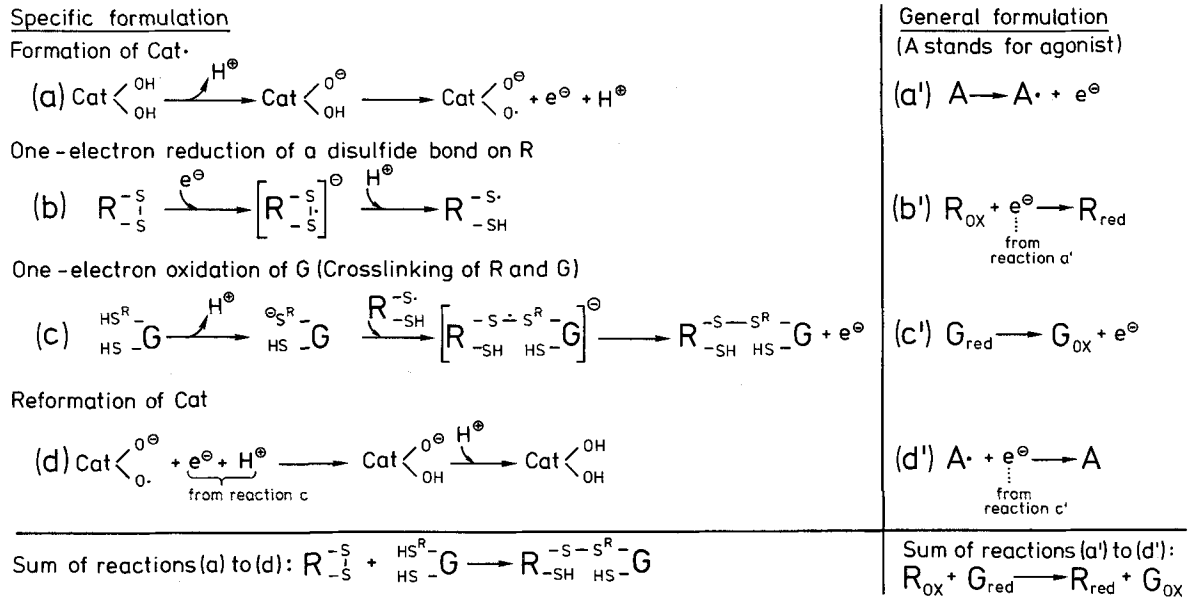
Desensitization

In β-adrenoceptor systems two types of desensitization, i.e. reduced responsiveness to hormone stimulation, can be distinguished: a) a *quasi-permanent* (long-term or chronic) type which is observed only after prolonged exposure to agonist, often accompanied by a loss of ligand-binding sites due to internalization, and which is



A hypothetical model of catecholamine action in the β-adrenoceptor system. Part A: R and G are depicted as the left- and right-hand partners, respectively, at each step of the cycle. Cat and Cat · stand for catecholamine in the hydroquinone and semiquinone states, respectively. S · is a short-lived thiyl radical produced by scission of a disulfide bond. Ia, Ib, II and III represent different states of R, G-interaction during the cycle. The ovals, rectangles and circles symbolize different conformational states of R and G accompanied by intermediate, high and low agonist affinity of R, respectively. The components in the reduced state are indicated by double contours. Two critical SH-groups are assumed to be present on G as may be concluded from experiments with N-ethylmaleimide (see, for instance, André et al.[3]). Interaction of G with adenylate cyclase is not considered in the scheme. For a detailed description see the text. Part B: In accordance with the known pH-dependence of the action of catecholamines[8, 10, 13, 15, 52] and of disulfide-thiol interchange reactions[27, 55, 63] as well as with a pK-value of 3.7 of the phenolic OH-group of adrenaline semiquinone[48] the anionic species (catecholate, thiolate, catecholate radical) are regarded as the true reactants. The sum of reactions (a) to (d) corresponds to the forward reaction of equation (2); in the general formulation the sum of reactions (a') to (d') is identical with the forward reaction of equation (1). The specific scheme, represented by reactions (a) to (d), does not intend to exclude the possibility that the electron transfer from Cat to the disulfide group of R and the electron back-transfer from the R, G-crosslinking disulfide group to Cat · occur indirectly (e.g. via aromatic amino acid residues or metal ions) rather than directly.

Ⓑ Individual reactions during transition from state Ia to state II



slowly reversible; b) a *transient* (short-term or acute) type which is established within seconds or at least 1–2 min, is not accompanied by a loss of ligand binding sites, and is rapidly reversible. Common to both types of desensitization is a greatly reduced affinity for agonists. A great number of experimental findings (for a review see Perkins et al.[46]) have led to the generally accepted notion that a desensitized state is characterized by 'uncoupling' of G and R.

The question now arises: how can the described desensitization phenomena be correlated with the proposed redox model? Actually, the specific model according to the figure suggests a new way to understand desensitization in molecular terms: 'uncoupling' can be viewed as the prevention of formation of a disulfide bond between R an G. I suggest that this could be achieved by raising the pK-values of the cysteinyl residues involved by nearby quasi-phosphorylation (binding of GTP to G) or true phosphorylation (covalent binding of a phosphate group to R and/or G). According to this suggestion the former process leads to short-term densensitization which is rapidly reversible, whereas the latter process leads to long-term desensitization which is only slowly reversible. This concept is supported by the recent finding that R of turkey erythrocytes is indeed phosphorylated after long-term desensitization[31, 45, 54, 59]. It remains to be seen whether phosphorylation of R after long-term desensitization is a general characteristic of β-adrenoceptor systems. In this context, another phenomenon which has been long known[8, 13, 52] and recently reinvestigated[10, 15] appears to be significant: acidification of the blood results in a markedly decreased activity of catecholamines. In the frame of the proposed redox model involving a disulfide-thiol interchange the desensitizing effect of acidosis

can be easily explained by the fact that the rate and extent of disulfide-thiol interchange reactions decrease with decreasing pH[27, 55, 63].

Concluding remarks

There is no doubt that thiol and disulfide groups play an eminent role in many membrane-related processes. Robillard and Konings[49] recently attempted to integrate a large body of experimental data on solute transport and energy transduction into a unifying hypothesis involving dithiol-disulfide interconversion. A basic feature of the Robillard-Konings hypothesis is that the conversion of a ligand binding site from a high-affinity to a low-affinity state and vice versa is mediated by a change of the redox state of vicinal thiol groups. Robillard and Konings mentioned that their hypothesis may be applicable also to hormone-receptor interactions but they did not treat these interactions in any detail.

In this paper, I have formulated a hypothetical model which implies that a disulfide-thiol exchange reaction plays a key role in the mechanism of β-adrenergic stimulation. In view of a wealth of experimental data it is beyond doubt that sulfhydryl groups are indeed of crucial importance in the activation mechanism of β-adrenoceptor agonists. However, some specific features of the proposed model, especially the transient cross-linking of R and G by a disulfide bond and the involvement of catecholamine semiquinone radicals, are at the present time largely speculative. Clearly, further experimental work is necessary to test the model in its general and specific aspects.

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