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Trigger Meetings

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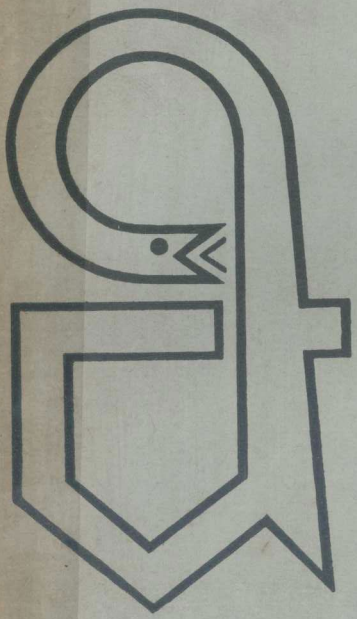

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Contents

Trigger Meetings

Biochemical Determination of the Receptor Action

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I. Introduction

This lecture deals with the biochemistry of chemopharmacodynamic action. Our definition of a chemopharmacodynamic action is a chemical stimulus which causes a chemical or physicochemical reversible change in the cell membrane *without influencing the cell's electrical properties* or alternatively the change in the electrical properties is a consequence of the stimulus-response phenomenon and *not the cause of it*. We shall call the mechanism which conveys to the cell interior the information that such stimulus did happen: Receptor Mechanism.

A part of this mechanism is the target area (or as it is called, the Receptor). The end result of activation of the receptor mechanism is a change in the concentration of a particular biochemical constituent which couples (hence the name Coupler) the stimulus to the response. In the meantime Ca^{++} ions, Na^{+} ions and cyclic AMP were implicated in different tissues as couplers (Fig. 1).

Table 1 summarizes some properties of the stimulus-response phenomenon as opposed to recovery. The results in Table 1 were obtained mainly on two models: the melanophore reaction of Hyla frog skin and the potassium depolarised ileal muscle of rat. Further examples of chemopharmacodynamic action (or as alternatively called: pharmacomechanical coupling) is given in the literature [31]. All the models suffer from the drawback of being multicellular, multilayer systems, composed of more than one type of cell [5].

I wish to introduce you to two new models, hitherto sparingly used for biopharmacological studies, namely the endothelial layer of the rabbit cornea, and the Vorticella. The endothelium of the cornea is an excellent model for transcellular, active transport of isotonic salt

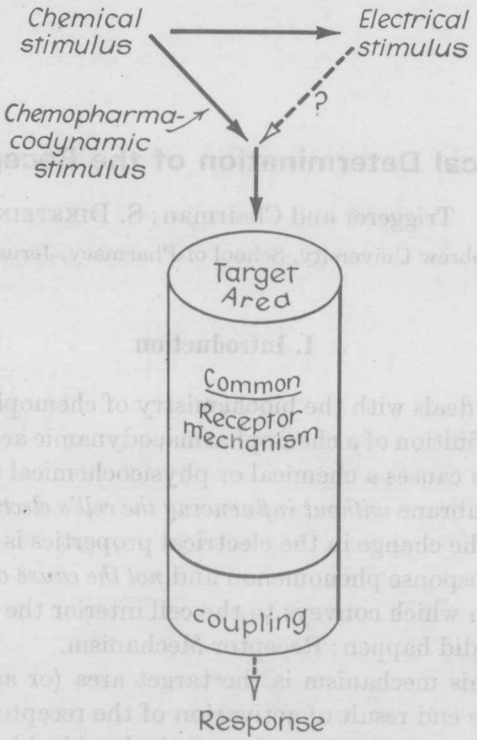

Fig. 1. Chemopharmacodynamic action and the receptor mechanism.

solution which has some properties in common with recovery. The Vorticellidae are protozoa with contractile stalks. In a way it reminds one of a very primitive muscle cell. The mechanism of contraction (response) is different, however [26].

Before bringing you the results, I wish to familiarize you with the techniques involved, as I hope that these models will rapidly enter into the family of the accepted ones.

A cross section of the cornea of the rabbit is shown in Fig. 2. The thickness of the stroma is determined by two opposing forces: on the one side, the swelling pressure of the stroma, which tends to suck in water (and thus increase the thickness) through the endothelium, and on the other side, the endothelial pumping system, which pumps isotonic or nearly isotonic water out of the stroma [10]. The two fluxes are balanced in the normal cornea [20]. By cooling the eye the endothelial pump is inhibited, thus the cornea swells. During a period of 24 h at 4° C it will swell from 0.35 mm to 0.55 mm or so. If we now mount the cornea with silicon oil over the epithelium to prevent water loss, and perfuse the endothelium at 37° C

Table 1

Metabolic properties of the stimulus-response-recovery cycle

Stimulus-response	Recovery
Small Q_{10}	Large Q_{10}
Movement of ions and/or fluid according to gradient	Movement of ion and/or fluid against gradient
Does not require metabolism	Requires continuous metabolic energy supply which is blocked by antimycin and flavoprotein electron flow inhibitors
Induced by agents capable of oxidising flavoproteins and/or SH groups	Induced by agents capable of reducing flavoproteins and/or SH groups
Cardiac glycoside in low concentration is ineffective or slightly stimulates	Cardiac glycoside in low concentration very effectively inhibits
Not blocked by actinomycin or puromycin	Not blocked immediately by actinomycin or puromycin, but it can only be sustained for a shorter time in their presence
Probably requires calcium ions	Probably requires sodium ions

we can study the metabolic requirements of the pump by monitoring the thickness of the cornea. Unless very complicated perfusion fluids are used the endothelial cells will not pump in Krebs-Ringer for more than 3–4 h (Fig. 3). By a stroke of luck we found that the addition of glutathione (but not cysteine) to a solution similar to Krebs-Ringer bicarbonate increases the pumping efficiency of the preparation and its survival to 6 h or more, thus making it comfortable [21]. There is no electrical potential difference through the actively working endothelium. The endothelium, as expected, contains a powerful Na^+, K^+, stimulated ATP-ase, which is ouabain sensitive [28].

The Vorticellidae contain two main groups: the Carchesium, which are colonial and the solitary Vorticellae. Although no difference has been found in our laboratories as far as any stimulus-response is concerned, we prefer the Vorticella since, in a way, it has a simpler structure and the stalk is always contractile, whereas in the Carchesium only the young with 3–4 heads have contractile stalks. Histochemically, the stalk does not contain free lipids or phospholipids [27], but does contain an ATP-ase and is rich in SH groups [17, 18]. The lack of free lipids probably could mean less complications for penetration, but there is no data available on this subject. The stalk which might reach 1 mm in lenght and about 20 μ in diameter contains the contractile myoneme in a passive health [26]. The contraction is dependent on Ca^{++} ions [11]. The usual stimulus which has been applied is touch or electricity, which is followed by a *single* contraction for about 0.2 sec, then a much slower relaxation. The main observation made in our laboratory, which permits its use as a model for chemopharmacodynamic stimulus, was that certain chemicals cause *rhythmic* contraction-relaxation cycles, which persist for some time even in 'isotonic 0.01 M K_2SO_4. I hasten to add that it is not known

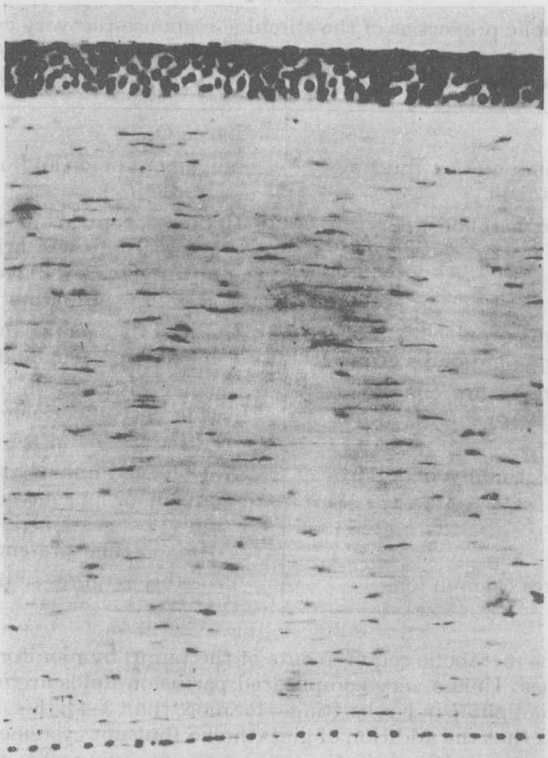

Fig. 2. Cross section of a rabbit cornea.

what the exact ionic composition of the cell is. The size of the cells, their simplicity, the fact that they are already fixed at one end, and the possibility of unlimited supply, strongly favour them as a model.

Results

Endothelial Transport in Cornea

As I pointed out, glutathione and bicarbonate buffer are essential for survival. As energy sources, only glucose, fructose and adenosine, but not desoxyadenosine, were found to support the pump. Adenosine is the best substrate. All the intermediates of glycolysis, Krebs-cycle intermediates, fatty acids and amino acids are inactive [21]. The active transport is inhibited by the following poisons:

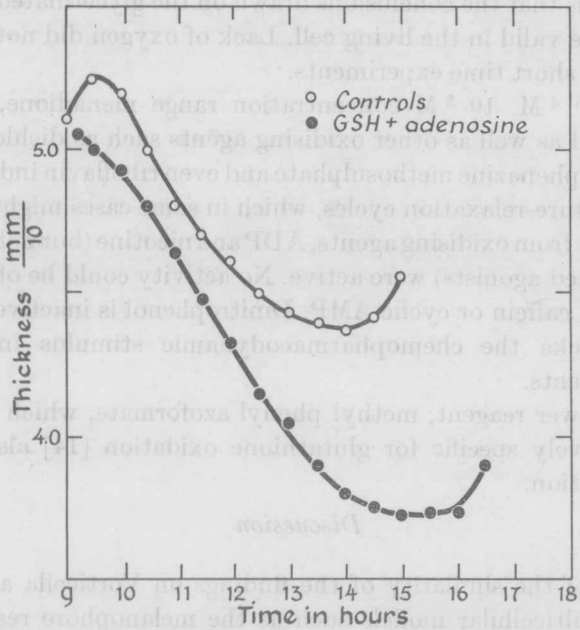

Fig. 3. Effect of glutathione on the endothelial pump.

Ouabain (10^{-7} M), KCN (10^{-5} M), 2-desoxyglucose (5, 10^{-3} M), Na benzoate (2, 10^{-4} M), antimycin A (10^{-9} M), oligomycin (10^{-7} M) and iodoacetate (2, 10^{-4} M).

The endothelial pump is very sensitive towards oxidising agents. Menadione 10^{-5} M rapidly inhibits it. The pump is exclusively dependent on the presence of Na^+ ions; Li^+, $Tris^+$ or sucrose were unable to replace it. This is similar to other models for the active transport of isotonic water. The Kosower reagent, methyl phenyl azoformate, induces a reversible swelling. This reagent is claimed to be relatively specific for glutathione [14].

Vorticella

As with all other models, it was found that the contracture time, as measured by cinephotography, is changed relatively little within the temperature limits of 10–37° C being 0.2–0.3 sec, but the relaxation time is varied from 0.5 sec up to 10 sec in the cold. Due to this observation,

it seems to us that the conclusions drawn on the glycerinated stalk [11] might not be valid in the living cell. Lack of oxygen did not have any effect in the short time experiments.

In the 10^{-4} M, 10^{-5} M concentration range menadione, dehydroascorbic acid as well as other oxidising agents such as dichlorophenolindophenol, phenazine methosulphate and even riboflavin induce rhythmic contracture-relaxation cycles, which in some cases might go on for hours. Apart from oxidising agents, ADP and nicotine (but not any of the other accepted agonists) were active. No activity could be observed by theophyllin, caffein or cyclic AMP. Dinitrophenol is inactive. Ouabain 10^{-4} M blocks the chemopharmacodynamic stimulus induced by oxidising agents.

The Kosower reagent, methyl phenyl azoformate, which is claimed to be relatively specific for glutathione oxidation [14] also induced rhythmic action.

Discussion

Because of the similarity of the findings on Vorticella and on our previous multicellular models such as the melanophore reaction and depolarised smooth muscle, we feel it to be justified to assume that a non-specific part of the receptor mechanism is common to all cells (Fig. 1). Common mechanisms, centering around the sodium pumps, were also suggested for the active transport of *any* substance into and out of the cell [4] and for the transcellular transport of *any* substance in the case of the intestinal transport [3].

The first obvious question is, how can one compare an essentially intracellularly directed phenomenon, as the Vorticella contracture or other receptor phenomenon, with a transcellularly directed, active transport of isotonic water, as presented by the cornea ? The answer is, that they have, surprisingly, many properties in common. These common properties are: the involvement of glutathione and the effect of oxidising agents, especially those of menadione and the Kosower reagent, the probable involvement of ADP, the effect of ouabain, lack of immediate sensitivity to oxygen, and the temperature dependence.

It seems to me that the similarities are too numerous to discard them as accidental. This being the case, then either we are observing a basic structural property of the living cell or cell membrane, or we are studying clues for the interaction between the biochemistry of the cell

and physiological functions not involving electrical phenomenon. Mainly, because of the reversibility of the glutathione oxidation effect and because of the cyclic nature of the Vorticella contracture, I believe in the second alternative.

If, indeed, we are studying the interaction between biochemistry on the one side and physiology-pharmacology on the other, then the next question is: where is the probable interaction on the metabolic map?

The lack of immediate effect by lack of oxygen excludes the mitochondrion and the oxidative phosphorylation path as the actual gearbox (although it can still be the main source of energy). The effectivity of adenosine (which can supply both NADH and NADPH) as compared to the lack of effectivity of desoxydenosine (which can supply only NADH [30]), the fact that oxidising agents such as menadione are known to stimulate the pentose shunt [12], the dependence of glucose-6-phosphate dehydrogenase activity on glutathione reductase activity [2], and on the ATP/ADP ratio [1], stimulation of the pentose shunt by desoxycorticosterone acetate [19]; all of them point directly towards the importance of a part of the pentose shunt including the glucose-6-phosphate dehydrogenase and indirectly to those enzymes, which handle TPNH.

The importance of the pentose shunt in general physiological-pharmacological phenomena is not a new idea. It was advocated to explain smooth muscle action and the action of some tranquillizing drugs [16], it was suggested to be the receptor point for ACTH action in steroidogenesis [22], it was found to be inhibited by aldosterone in the toad bladder and was thought to be somehow connected with its effect on the electrogenic sodium pump [13], it was found to be activated by multiple electrical stimulation of the crustacean stretch receptor [9], and thought to be connected with the phagocytosis [34] and the cataract formation [32], just to mention a few examples.

Of course, the importance of a part of the pentose shunt is by no means the only explanation. The alternative would be a specific ATP-ase [7] which is known to be sensitive to oxidising agents [33]. In this case, the effect of the metabolic precursors would be reflected on their ability to preserve the proper ratio reduced to oxidised glutathione, needed for the preservation of the ATP-ase sulphydryl groups [15].

In essence, the question is whether in the case of chemopharmacodynamic

action an ATP-ase 'greases' a component of the pentose shunt 'gear' or the pentose shunt 'greases' an enzyme, for example an ATP-ase 'gear'.

The last question is, what could be the common *physiological mechanism* between a Stimulus-Response-Recovery cycle and a transcellular phenomenon? I believe the clue is given by the observation, that in the Stimulus-Response-Recovery models excessive chemopharmacodynamic action will usually produce inhibition in the normal 'recovered' state rather than in the 'responsed' state. Our working hypothesis is similar to that advanced for the mitochondrial shrinking-swelling, namely we deal with an induced oscillatory phenomenon [24]. The induced oscillatory reaction, in different phases, works either as an ion gate as described for K^+ in other models [8, 23, 25, 29], in the case of Vorticella contracture, or as an active translocator mechanism in the case of Vorticella relaxation or corneal shrinking. Disrupting the oscillation by an excessive interaction will immobilize the 'gate' and prevent any further physiological phenomenon.

Summary

Unicellular models are advocated for the study of mutual interaction between the biochemistry of the cell and a physiological task of the cell *not* involving electric potential. For drug induced stimulus the cyclic contracture of the Vorticella stalk seems to be a proper model and for the active transport of isotonic water the endothelial layer of the cornea is used.

It was found that both models depend on glutathione. Because of the reversibility of the probably selective glutathione oxidation, I favour the concept that it is involved in the biochemical-physiological mechanism, rather than being a simple building block of the cell membrane. It was also reasoned that part of the pentose shunt is much more important in its immediate effect than any other main metabolic pathway. Because of the effect of ADP or ATP generating reactions, we cannot rule out the involvement of an ATP-ase. The question is whether an ATP-ase together with the glutathione reductase governs the pentose shunt or the pentose shunt through keeping the glutathione in the reduced state governs an enzyme—for example an ATP-ase or 3-phosphoglycerate dehydrogenase.

It is hoped that the study of the interaction of multienzyme systems