SHORT COMMUNICATIONS

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Affinity oxidation of the reduced acetylcholine receptor

The depolarizing response of the isolated electroplax of *Electrophorus electricus* to acetylcholine and to other monoquaternary ammonium activators is inhibited following a 10-min exposure of this cell to 1 mM dithiothreitol[1]. This inhibition is completely reversed by a 10-min application of oxidizing agents such as 1 mM 5,5'-dithiobis-(2-nitrobenzoate) or 1 mM potassium ferricyanide. In addition, a 20-min application of 5 mM D- or L-cysteine reverses the effects of reduction by dithiothreitol. Neither a saturated solution of cystine (approx. 0.4 mM) nor 5 mM β-mercaptoethanol nor 5 mM reduced glutathione, applied 20 min, have any effect on the inhibition due to dithiothreitol. It was concluded that dithiothreitol reduces a disulfide bond on a component involved in the response to acetylcholine; that the oxidizing agents cause reformation of the disulfide, and that cysteine in an unknown way also can participate in the reoxidation of the reduced disulfide. The latter interpretation is supported by the report that cysteine can catalyze the air oxidation of dithiothreitol in solution[2].

Further evidence suggested that the component whose reduction and reoxidation is reflected in changes in the response of the electroplax is the acetylcholine receptor and that the disulfide susceptible to reduction is close to the binding site for acetylcholine on the receptor. Quaternary ammonium maleimide derivatives apparently alkylate the reduced component three orders of magnitude faster than uncharged maleimides[3,4]. The reactions of some of the quaternary ammonium alkylating and acylating agents with the reduced component result in a depolarizing response which can not be reversed by washing but can be temporarily reversed by high concentrations of reversible competitive inhibitors such as (+)-tubocurarine; after the reversible inhibitor is washed out, the response reoccurs[5]. Furthermore, the effect of reduction is to change the specificity of the receptor; *e.g.* the response to carbamylcholine is inhibited, but hexamethonium, normally a competitive inhibitor, becomes an activator[3,4]. Hence, the quaternary ammonium alkylating and acylating agents appear to be affinity labels of the reduced receptor, binding reversibly to the negative subsite of the receptor and reacting covalently with a nearby sulfhydryl group. We report the effects of cholinethiol and of related quaternary ammonium thiols and bisquaternary ammonium disulfides on the reduced receptor. These compounds appear to interact with the unmodified receptor, activating in the case of the thiols and competitively inhibiting in the case of the disulfides[6,7].

We find that 1 μM cholinethiol (2-mercaptoethyltrimethylammonium iodide) applied 5 min completely reverses the inhibition of the response of the electroplax to carbamylcholine due to dithiothreitol (Fig. 1). *S*-Methylcholinethiol, like cholinethiol a depolarizing agent, has no effect on the inhibition due to reduction (Fig. 1).

The response is also restored by micromolar concentrations of homocholinethiol, cholineselenol, and the tertiary analog of cholinethiol (2-dimethylaminoethanethiol) which is positively charged at pH 7.0. These quaternary ammonium thiols, therefore,

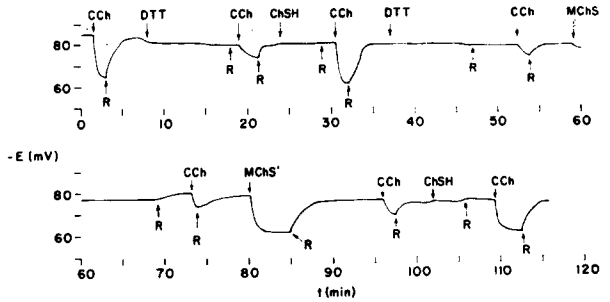

Fig. 1. Inhibition by 1 mM dithiothreitol (DTT) of the response of the electroplax to 40 μM carbamylcholine (CCh); restoration of response by 1 μM cholinethiol (ChSH) but not by *S*-methylcholinethiol (MChS, 10 μM; MChS', 100 μM). R: 165 mM NaCl, 2.3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.2 mM K_2HPO_4, 0.3 mM KH_2PO_4, 10 mM glucose, pH 7.1. Dithiothreitol was dissolved in a Ringers solution identical to R except that phosphate is replaced by 2 mM Tris (pH 8.0). Electroplax were dissected and mounted in a two chamber holder as previously described[8,9]. The potential difference between an interval glass microelectrode and an external electrode on the innervated side of the cell is recorded.

reverse the effects of reduction at concentrations several 1000-fold lower than that at which cysteine is effective (*e.g.* 1 μM cholinethiol applied 5 min compared with 5 mM cysteine applied 20 min). Cholinethiol also causes complete reversal of the effects of reduction at a 10-fold lower concentration than that at which it causes an appreciable response of the unreduced electroplax. The ability of cholinethiol to reverse the effect of reduction is retarded if it is added in the presence of hexamethonium (Fig. 2).

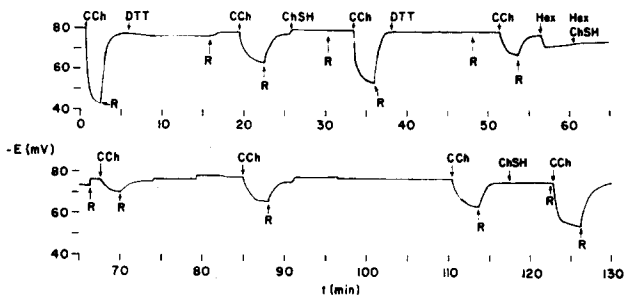

Fig. 2. Inhibition by hexamethonium of the reversal by cholinethiol. The addition of 2 μM cholinethiol (ChSH) subsequent to 1 mM dithiothreitol (DTT) restores the response to 40 μM carbamylcholine (CCh). Following a second application of dithiothreitol, cholinethiol in the presence of 1 mM hexamethonium (Hex) fails to restore the response, even after prolonged washing to ensure removal of hexamethonium. After washing, a subsequent application of cholinethiol restores the response.

The disulfides of cholinethiol and homocholinethiol completely reverse the effects of reduction in the concentration range of 0.2–0.5 μM applied 5 min and are thus even more effective than the thiols. In addition these bisquaternary ammonium

disulfides share a property with hexamethonium[3] that they are reversible competitive inhibitors of the unmodified receptor and activators of the reduced receptor. This is illustrated in Fig. 3, which shows an experiment in which much higher concentrations of cholinedisulfide than are needed for reoxidation are added following dithiothreitol, and a transient response is obtained. Cholinedisulfide activates the reduced receptor but also reoxidizes it to the native form which is not activated by cholinedisulfide. It is unlikely that cholinethiol is acting through its conversion to the disulfide since freshly made solutions of cholinethiol contained no detectable disulfide, and its rate of autooxidation at pH 7.0 is very slow (approx. 10 % per day).

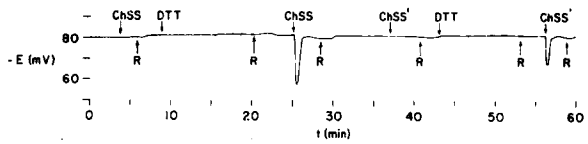

Fig. 3. Transient depolarization of the dithiothreitol (DTT)-treated electroplax by cholinedisulfide (ChSS, 100 μM; ChSS', 50 μM).

The reoxidation of the reduced receptor by cholinedisulfide and its analogs and by 5,5'-dithiobis-(2-nitrobenzoate) probably proceeds by formation of a mixed disulfide followed by exchange[10,11] to yield the original disulfide bridge[12,13]. The mechanism whereby cysteine and cholinethiol and its analogs reverse the reduction is not known. The thiols themselves are not oxidizing agents but must in some way catalyze the transfer of electrons to oxygen, perhaps through a partially oxidized intermediate such as the sulfenic acid derivative. This derivative could form a mixed disulfide with a receptor sulfhydryl followed by an exchange yielding the original disulfide and the thiol. On the basis of the several 1000-fold increase in the rate of reoxidation of the reduced receptor due to the quaternary ammonium moiety, it appears likely that the bisquaternary ammonium disulfides and the effective derivatives of the quaternary ammonium thiols are acting as affinity oxidizing agents, binding reversibly to the negative subsite of the receptor and reacting with the nearby receptor sulfhydryls. As with the quaternary ammonium alkylating agents[3,4], the reaction of the reduced receptor with the postulated effective derivative of cholinethiol is retarded in the presence of a reversible ligand of the receptor, hexamethonium, whereas the reaction with 5,5'-dithiobis-(2-nitrobenzoate) and with the postulated effective derivative of cysteine is not affected by hexamethonium.

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Modification of membrane composition in growing photosynthetic bacteria

Synthesis of energy-converting membranes in purple bacteria growing photosynthetically appears to be regulated in some way by the average energy flux[1,2]. Growth rate is, of course, also related to energy flux and, consequently, membrane synthesis and growth rate are ordinarily interdependent. Thus, in typical purple bacteria there is an inverse relation between growth rate (divisions/h) and quantity of membrane per unit of dry weight[2,3]. It has been suggested[2] that this results from operation of a "compensatory" control system, which, in effect, aids the photosynthetic cell in maintaining its chemical energy flux when the light intensity changes. Perhaps the most common situation encountered in this respect is the decrease in light intensity that inevitably occurs as the density of cells increases in a growing batch culture (usually referred to as "self-shading"). When the continuous light intensity diminishes beyond a certain point, the growth rate decreases and, per unit of dry mass, the quantities of bacteriochlorophyll and membrane increase[4]. This also occurs when cultures are exposed to saturating, but intermittent, light[1]. These two situations are probably closely related in that momentary self-shading of motile cells is, in effect, intermittent illumination. The physiological effects ensuing from exposure of cells to bright intermittent or dim continuous light have been interpreted[1] as regulatory responses initiated by conditions of energy stress.

The production of "additional" membrane as a response to energy stress might be viewed as a kind of "differentiation" of the cytoplasmic membrane. Experimental attempts with various photosynthetic bacteria to detect differences in chemical composition between the cytoplasmic and "additional" membrane, however, have given contradictory data[3]. In this communication, we present evidence for a chemical differentiation, in respect to phospholipid composition, of "additional" membrane in the bacterium *Rhodopseudomonas capsulata*.

R. capsulata (strain "St. Louis", American Type Culture Collection No. 23782) was grown in a synthetic medium (initial pH 6.8) containing 0.4% DL-malic acid,