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INACTIVATION OF THE β-ADRENERGIC RECEPTOR IN SKELETAL MUSCLE BY DITHIOLS*

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Abstract—The effect of sulfhydryl compounds on binding of the β-adrenergic antagonist (−)-[^{3}H]dihydroalprenolol [(−)-[^{3}H]DHA] to a microsomal fraction from rabbit skeletal muscle was examined. Inhibition of binding by a variety of adrenergic agonists and antagonists and the effects of these agents on adenylate cyclase were consistent with the β-adrenergic receptor in this tissue being of the β_2-subtype. Binding of (−)-[^{3}H]DHA was reduced by incubating the membranes with dithiols such as dithiothreitol (DTT), 1,3-dimercapto-2-propanol and 1,4-dimercaptobutane; monothiols were much less potent. DTT-induced decline in (−)-[^{3}H]DHA binding resulted primarily from a decrease in receptor number. Inactivation was partially reversed by the oxidant H_2O_2. Binding sites could be locked in the inactivated state by incubating DTT-treated membranes with the alkylating agent iodoacetamide. Both β-adrenergic agonists and antagonists protected against inactivation. Adenylate cyclase activity in the membranes was increased by DTT. The enzyme was rapidly inactivated by H_2O_2, and this could be partially reversed by DTT. It is concluded that the β-adrenergic receptor of skeletal muscle contains an essential disulfide moiety which can be inactivated by reducing dithiols. Adenylate cyclase, on the other hand, contains at least one essential sulfhydryl which is preserved by dithiols.

Recent studies suggest that the β-adrenergic receptor contains a disulfide moiety which is essential for binding of adrenergic ligands and which can be reduced by dithiols such as DTT§. In turkey erythrocyte membranes, which possess an adrenergic receptor of the β_1-subtype, DTT has been shown to cause a decrease in the number of binding sites for the radioligand (−)-[^{3}H]DHA without affecting affinity [1]. The dithiol has also been shown to cause a reduction in specific (−)-[^{3}H]DHA binding in particulate fractions from C6 glioma cells [2], but in this case the effect was due to a decrease in receptor affinity with no loss in receptor number. DTT does not seem to affect (−)-[^{3}H]DHA binding to the β_2-adrenergic receptor in frog erythrocyte membranes [3]. Adenylate cyclase, to which the β-adrenergic receptor is coupled in many cell types, seems to contain essential sulfhydryl groups. DTT is used frequently to preserve enzyme activity.

In skeletal muscle, adenylate cyclase appears to be coupled to an adrenergic receptor of the β_2-subtype [4–6]. In this study we have examined the effect of reducing dithiols on the β-receptor in a "heavy" microsomal fraction from rabbit muscle.

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§ Abbreviations: DTT, dithiothreitol; Gpp(NH)p, guanyl-5′-yl-imidodiphosphate; and (−)-[^{3}H]DHA, *levo*-[propyl]-2,3-[^{3}H]dihydroalprenolol.

MATERIALS AND METHODS

[α-^{32}P]ATP (10–30 Ci/mmole), [2,8-^{3}H]cAMP (30–50 Ci/mmole), *levo*-[propyl]-2,3-[^{3}H]dihydroalprenolol (30–50 Ci/mmole) and Omnifluor were purchased from the New England Nuclear Corp., Montreal, Canada; Aqueous Counting Scintillant (ACS) was purchased from the Amersham Corp., Oakville, Canada. DTT, dithioerythritol, 2,3-dimercaptopropanol, Tris base, cAMP, ATP, GTP, phosphoenol pyruvate, pyruvate kinase (rabbit skeletal muscle Type II), 3-isobutyl-1-methylxanthine, sodium dodecyl sulfate, imidazole, (−)-isoproterenol, (+)-isoproterenol, (−)-epinephrine, (−)-norepinephrine, and (±)-propranolol were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. (+)-Norepinephrine was obtained as a courtesy from Sterling–Winthrop, New York, NY, U.S.A., as was (+)-alprenolol from AB Hässle, Goteborg, Sweden, butoxamine from Burroughs Wellcome, Greenville, NC, U.S.A., and metoprolol from Ayerst Laboratories, Montreal, Canada. Gpp(NH)p and 1,4-dimercaptobutane were purchased from ICN (Canada), Montreal, and 1,3-dimercapto-2-propanol from the Aldrich Chemical Co., Milwaukee, WI, U.S.A.

Preparation of microsomal membranes. Female rabbits (1.5 to 2 kg) were killed, leg muscles (gracilis, biceps femoris and semitendinosus) were removed and cleared of fat, and 60 g of tissue was homogenized in 5 vol. of 20 mM Tris–HCl, 1 mM EDTA, pH 7.5, for 3 min at maximum velocity in a Sorvall Omni-mixer at 4°. The homogenate was passed through a 1 mm^2 nylon mesh and centrifuged at 2000 *g* for 10 min. The supernatant fluid was strained

through twelve layers of cheesecloth and kept on ice. The pellets were homogenized in 3 vol. of buffer as above, and the supernatant fluid was recovered. This process was repeated a third time. The combined supernatant fluids were centrifuged at 38,000 *g* for 30 min. The resulting pellets were washed three times by suspension in 250 ml of 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.5 (buffer A), using a loose-fitting Teflon pestle and centrifuging at 38,000 *g* for 20 min. The residue was finally dispersed in 35 ml of buffer A in a Potter–Elvehjem homogenizer; the suspension which contained 5–6 mg/ml of protein was stored at −80°.

Binding of (−)-[^{3}*H*]*DHA*. The method of Mukherjee *et al.* [7] was used with modifications. Assays were performed in a system consisting of 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.5 (buffer A), together with 10 nM (−)-[^{3}H]DHA (1.5×10^5 dpm) and microsomal membranes (300–400 μg protein), final vol. 0.15 ml. The reaction was started by the addition of radioligand and was terminated after 10 min at 30° by dilution with 1.85 ml of cold buffer A. The diluted suspensions were immediately filtered under vacuum through Whatman GF/C glass fiber filters (24 mm diameter). The filters were washed with 20 ml of cold buffer A and dried for 1 hr at 60°, and radioactivity was determined by scintillation spectrometry using 7 ml of toluene containing 4 g/l of Omnifluor. Nonspecific binding was quantitated by conducting the assay in the presence of 10 μM (−)-alprenolol. Specific binding, which was 85–90% of total binding, was usually in the range of 60–90 fmoles/mg. All measurements were performed in triplicate.

Assay of adenylate cyclase. The method of Salomon *et al.* [8] was used. The assay mixture contained 50 mM Tris–HCl, pH7.5, 25 mM $MgSO_4$, 5.5 mM KCl, 1.0 mM 3-isobutyl-1-methylxanthine, 10 mM phosphoenol pyruvate, 170 μg/ml pyruvate kinase, 0.5 mM [^{32}P]ATP (40 dpm/pmole) and 50–200 μg membrane protein, final vol. 0.15 ml. The reaction was initiated, after 1 min equilibration at 30°, by the

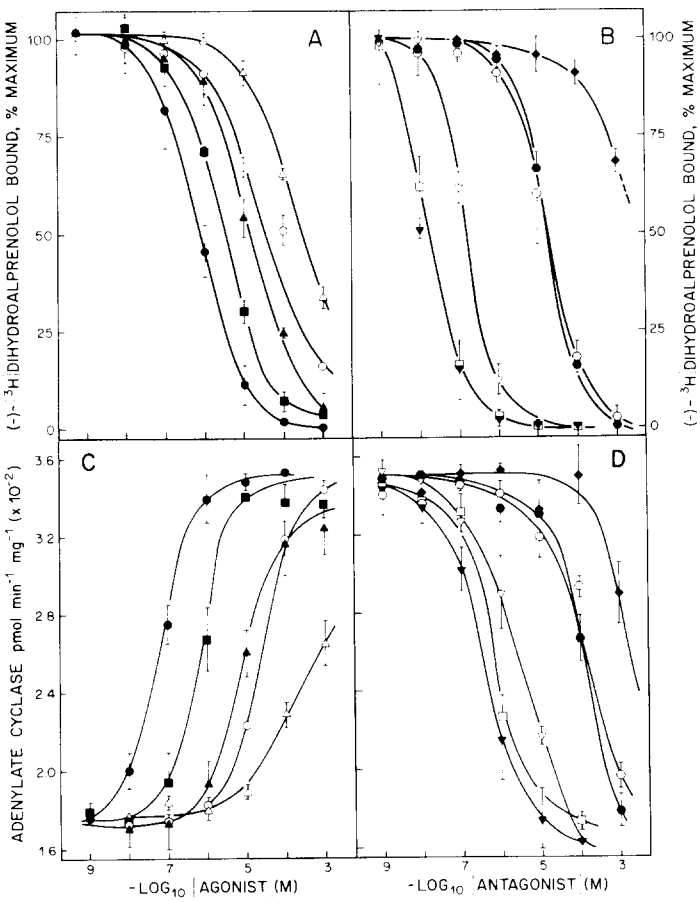

Fig. 1. Effects of adrenergic agonists and antagonists on specific (−)-[^{3}H]DHA binding and adenylate cyclase. Panels A and B: membranes were incubated under standard binding conditions with increasing concentrations of (A) agonists (●) (−)-isoproterenol, (■) (−)-epinephrine, (▲) (−)-norepinephrine, (○) (+)-isoproterenol, and (△) (+)-norepinephrine, and (B) antagonists (□) (±)-propranolol, (▼) (−)-alprenolol, (▽) (+)-alprenolol, (▬) butoxamine, (○) metoprolol, and (◆) phentolamine. Panel C: adenylate cyclase assays were performed in the presence of 100 μM Gpp(NH)p and various concentrations of agonists; symbols are the same as in A. Panel D: assays were performed in the presence of 100 μM Gpp(NH)p plus 10 μM (−)-isoproterenol and increasing concentrations of antagonists; symbols are the same as in B. Values are means ± S.E.M. of at least three separate experiments; all binding assays were performed in triplicate and enzyme assays in duplicate.

addition of membranes and was terminated after 10 min by the addition of 0.1 ml of "stop" reagent (2% sodium dodecyl sulfate, 40 mM ATP, 1.4 mM cAMP, pH 7.5) [8]. Fifty microliters of [^{3}H]cAMP solution (1×10^6 dpm/ml) was added to each tube to monitor recovery, and [^{32}P]cAMP was isolated [8] and quantitated by scintillation spectrometry using ACS mixture. All assays were performed in duplicate.

Protein was determined by the method of Lowry *et al.* [9].

RESULTS

Characteristics of (−)-[^{3}H]DHA binding. Specific binding of (−)-[^{3}H]DHA in the microsomal fraction was 6- to 7-fold higher than in the starting homogenate. Binding was proportional to protein up to 400 μg in the assay and reached equilibrium within 7 min at 30°. Addition of 10 μM unlabeled (−)-alprenolol, after maximum binding was achieved, resulted in rapid dissociation of bound (−)-[^{3}H]DHA. Specific binding was saturable at concentrations of (−)-[^{3}H]DHA below 10 nM. Scatchard analyses of binding data revealed two classes of binding sites, a high affinity site with a K_D of 1.1 nM and a lower affinity site with a K_D of 6.8 nM. From Arrhenius plots obtained from measurements of initial binding rates at several temperatures, the energy of activation (E_a) was calculated to be 42 kcal/mole.

Adrenergic agonists and antagonists competed with (−)-[^{3}H]DHA for specific sites in a dose-dependent manner (Fig. 1 A and B). Apparent dissociation constants (K_I) for ligands, estimated from concentrations which caused 50% inhibition of binding (using the equation $K_I = IC_{50}/(1 + [S])/K_D$, where S is the concentration of (−)-[^{3}H]DHA and K_D is the dissociation constant for this ligand obtained from Scatchard analyses), were in μM units: (−)-isoproterenol, 0.07; (−)-epinephrine, 0.27; (−)-norepinephrine, 1.23; (+)-isoproterenol, 4.25; (+)-norepinephrine > 30; and for antagonists, (±)-propranolol, 0.0015, (−)-alprenolol, 0.0012; (+)-alprenolol, 0.014; metoprolol, 1.47; and butoxamine, 1.47. Figure 1C shows dose-dependent stimulation of adenylate cyclase in the membranes in the presence of Gpp(NH)p; dose-dependent inhibition of 10 μM (−)-isoproterenol stimulation by antagonists is shown in Fig. 1D. The order of agonist potencies was similar to that seen for displacement of (−)-[^{3}H]DHA binding. Thus, the K_a values (μM) for agonists were: (−)-isoproterenol, 0.06; (−)-epinephrine, 0.78; (−)-norepinephrine, 9.3; (+)-isoproterenol, 26.0; and (+)-norepinephrine, 500. The K_I values for antagonists were calculated from the concentration required to cause 50% inhibition of (−)-isoproterenol-stimulated enzyme activity using the equation $K_I = IC_{50}/(1 + [S])/K_{act}$ where $[S]$ is the concentration of (−)-isoproterenol and K_{act} is the activation constant for this agonist. The values (μM) were: (±)-propranolol, 0.0033; (−)-alprenolol, 0.0016; (+)-alprenolol, 0.013; butoxamine, 0.72; and metoprolol, 0.87.

Effect of sulfhydryl compounds on (−)-[^{3}H]DHA binding. When membranes were incubated with a variety of sulfhydryl compounds, a dose-dependent decrease in (−)-[^{3}H]DHA binding occurred (Fig. 2). Of those agents tested, DTT (and dithioerythritol, data not shown) were the most potent; at 1 mM DTT only 50% of specific binding remained, and at 10 mM binding was completely eliminated. 1,3-Dimercapto-2-propanol was almost as effective as DTT, while 1,4-dimercaptobutane was less potent. 2,3-Dimercaptopropanol was 30-fold less effective and was similar to the monosulfhydryl glutathione. 2-Mercaptoethanol was the least potent, being 2 orders of magnitude less effective than DTT. The reducing agent $NaBH_4$ had no effect on binding at concentrations up to 20 mM. The action of DTT to reduce (−)-[^{3}H]DHA binding was both time and temperature dependent (data not shown).

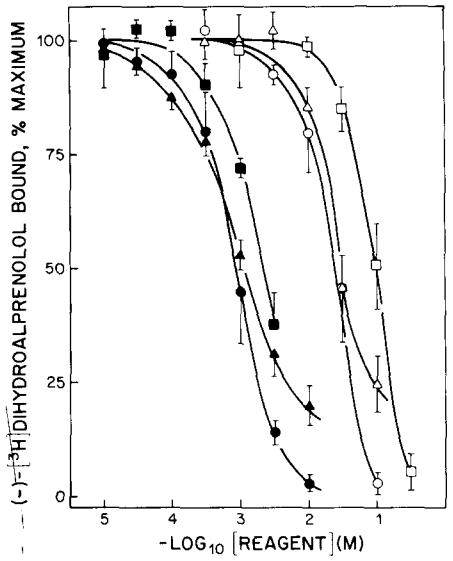

Fig. 2. Effect of sulfhydryl compounds on (−)-[^{3}H]DHA binding. Membranes were incubated for 15 min at 30° with increasing concentrations of sulfhydryl compounds immediately before the binding assay. Key: (●) DTT, (▲) 1,3-dimercapto-2-propanol, (■) 1,4-dimercaptobutane, (○) 2,3-dimercaptopropanol, (△) glutathione, and (□) 2-mercaptoethanol. Values are means ± S.E.M. of at least three experiments; each assay was performed in triplicate.

The effect of DTT on binding was examined in more detail. Membranes were incubated with 0.5 and 1.0 mM DTT prior to measurement of binding with various concentrations of (−)-[^{3}H]DHA. As can be seen in Fig. 3A, specific binding at saturation was reduced in a dose-dependent manner. Scatchard analyses of the data (Fig. 3B) revealed a decrease in binding site number and possibly a small decrease in affinity. From the slopes of the lines, K_D values of 1.15, 1.68 and 2.35 nM for control, 0.5 mM DTT- and 1.0 mM DTT-treated membranes, respectively, were calculated. Clearly the primary effect was a decrease in total binding sites. Competition for binding sites in membranes treated with DTT was also assessed. (−)-Isoproterenol and (−)-alprenolol competed for (−)-[^{3}H]DHA binding in DTT-treated membranes with the same affinity as that for control membranes (Fig. 4).

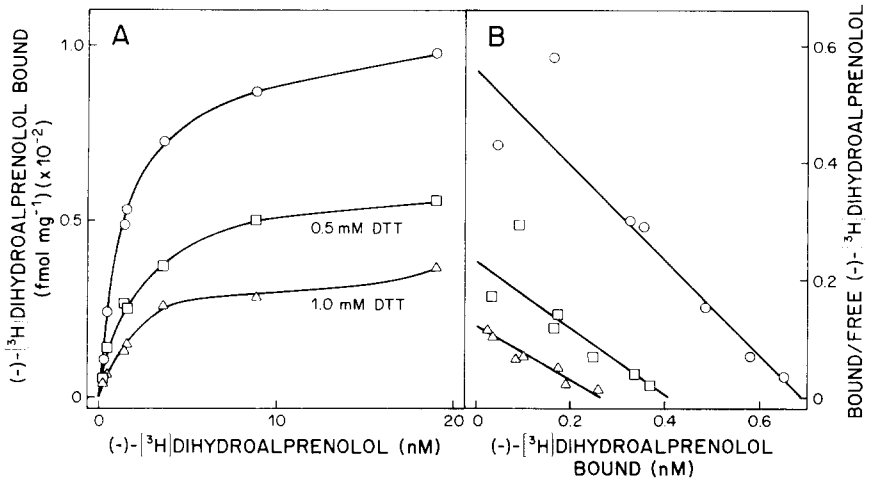

Fig. 3. Saturation of (−)-[^{3}H]DHA binding in membranes treated with DTT. Panel A: membranes were incubated with various concentrations of DTT for 15 min at 30° prior to measurement of binding. Data are representative of three similar experiments. Panel B: Scatchard plot of specific (−)-[^{3}H]DHA binding in membranes treated without (○) or with 0.5 mM (□) and 1.0 mM DTT (△).

Reversal of DTT-induced inactivation and "locking" of the inactivated state. Experiments were performed to see whether dithiol-induced inactivation could be reversed by oxidizing agents and whether the receptor could be locked in the inactivated (reduced) state by alkylation. The oxidant H_2O_2 and the alkylating agent iodoacetamide were selected since in control experiments they had no effect on binding in untreated membranes. Membranes treated with DTT were washed in buffer A, buffer A plus 20 mM H_2O_2, and buffer A plus 5 mM iodoacetamide followed by 20 mM H_2O_2 before analysis for

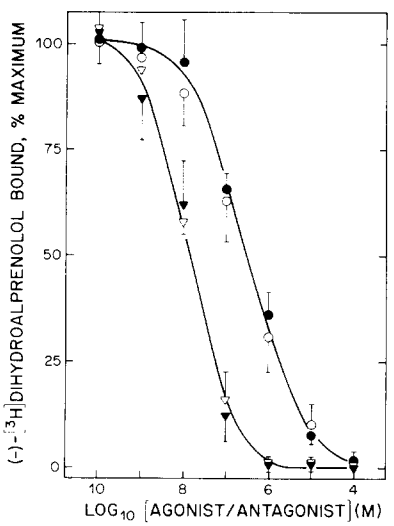

Fig. 4. Competition for (−)-[^{3}H]DHA binding in membranes treated with DTT. Membranes were incubated with 0.5 mM DTT for 15 min at 30°; binding measurements were then carried out in the presence of various concentrations of (−)-isoproterenol (●, ○) or (−)-alprenolol (▼, ▽). Closed symbols, control membranes; open symbols, membranes treated with DTT. Values are means ± S.E.M. of three separate experiments.

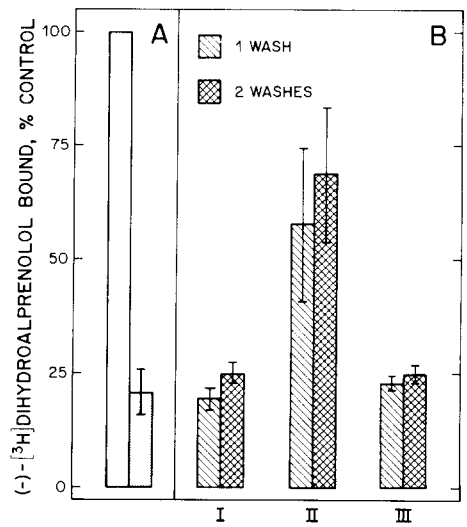

Fig. 5. Effect of iodacetamide and H_2O_2 on binding in DTT-treated membranes. Panel A: to 1 ml of membrane suspension (2 mg/ml) DTT was added to a final concentration of 2 mM and the suspension was incubated for 15 min at 30°. A similar suspension was incubated without DTT. Open bar, membranes without DTT; stippled bar, + DTT. Panel B: either iodoacetamide (5 mM) or H_2O_2 (20 mM) (final concentrations) was added to polycarbonate centrifuge tubes containing DTT-treated membranes, and the tubes were incubated for 5 min at 30°. A control tube contained membranes suspended in buffer alone. Buffer A (33 ml) containing the appropriate reagent was then added and the tubes were centrifuged at 38,000 *g* for 20 min. Pellets were washed once or twice in this manner, suspended in 1 ml of buffer A using a sintered glass homogenizer, and assayed immediately for (−)-[^{3}H]DHA binding. Bar set I, membranes washed in buffer alone; set II, treated and washed with H_2O_2; and set III, treated and washed with iodoacetamide followed by H_2O_2. The latter values were identical to membranes washed with iodoacetamide alone. Hatched bars, one wash; cross-hatched bars, two washes. Values are means ± S.E.M. of three separate experiments performed in triplicate.

Table 1. Effect of DTT in the presence of adrenergic agonists and antagonists*

Adrenergic ligand	Specific (−)-[^{3}H]DHA binding (fmoles/mg) −DTT	+DTT	Binding sites remaining (%)
None	90 ± 5	28 ± 5	31
Agonists			
(−)-Isoproterenol	32 ± 1	32 ± 1	100
(+)-Isoproterenol	84 ± 5	54 ± 2	64
(−)-Epinephrine	41 ± 1	33 ± 1	80
(−)-Norepinephrine	81 ± 3	50 ± 4	62
Antagonists			
(−)-Alprenolol	98 ± 1	98 ± 5	100
(±)-Propranolol	89	88	99

* Membranes were incubated for 5 min at 30° with 0.1 μM adrenergic agent in 100 μl prior to treatment with 2 mM DTT or with an equivalent volume of buffer for 15 min. Iodoacetamide was then added in 100 μl to a final concentration of 5 mM, and the incubation was continued for 5 min. Membranes were diluted with 35 ml of buffer A containing 5 mM iodoacetamide and sedimented at 38,000 *g* for 20 min. Washing was repeated in this manner three more times. Pellets were then suspended in a sintered glass homogenizer and assayed for (−)-[^{3}H]DHA binding. Values are the means ± S.E. of three experiments, each determination performed in triplicate.

(−)-[^{3}H]DHA binding. Washing treated membranes with buffer alone resulted in a small recovery of binding capacity (Fig. 5). When H_2O_2 was present, significant but not complete recovery occurred. When iodoacetamide was present, absolutely no recovery of binding was seen, and subsequent washing of these membranes with H_2O_2 failed to restore binding. Identical results were obtained when membranes were treated with 1,3-dimercapto-2-propanol as the reductant.

Protection of binding sites from DTT inactivation by adrenergic agents. When membranes were incubated with agonists or antagonists prior to treatment with DTT, (−)-[^{3}H]DHA binding sites were protected from inactivation (Table 1). β-Adrenergic specificity was observed in that the potency order was (−)-isoproterenol > (−)-epinephrine > (−)-norepinephrine, and (−)isoproterenol was more effective than the (+)-isomer. Alprenolol and propranolol also afforded protection. In membranes treated with agonists, subsequent binding of (−)-[^{3}H]DHA was reduced (Table 1). In each case the percentage of binding remaining following DTT treatment was calculated on the basis of the appropriate value in the absence of DTT.

Effect of DTT and H_2O_2 on adenylate cyclase. The effect of DTT on adenylate cyclase in membranes (prepared in the absence of the dithiol) is shown in Fig. 6A. DTT at concentrations as low as 0.5 μM caused an increase in basal, as well as in Gpp(NH)p- and Gpp(NH)p plus isoproterenol-stimulated, activities. H_2O_2 caused a sharp decline in these variables (Fig. 6B), and this was partially reversed by subsequent addition of DTT (Fig. 6C).

DISCUSSION

Specific binding of (−)-[^{3}H]DHA to membranes of the "heavy" microsomal fraction of rabbit muscle displayed characteristics analogous to those we have reported for plasma membranes from this tissue [5]. The microsomal fraction, although less pure, required much less time for preparation, and nonspecific binding was less. In the microsomal fraction, nonspecific binding ranged between 10 and 15% of total binding; in plasma membrane preparations [5] it constituted 25–30% of total binding. The plasma membrane preparation we use employs extraction of homogenates with high concentrations of LiBr and KBr [5] which may result in increased availability of nonspecific sites. The data on specificity and potencies of adrenergic agonists in competing for (−)-[^{3}H]DHA binding sites correlated well with their order of potencies for stimulating adenylate cyclase. There was also good correlation between the ability of antagonists to inhibit binding and isoproterenol-stimulated adenylate cyclase. Agonist action was stereospecific in that (−)-isomers were much more potent than (+)-isomers. These data extend existing evidence [4–6, 10] that, in skeletal muscle from several species, adenylate cylase is coupled to an adrenergic receptor of the β_2-subtype. The use of the specific β_1-antagonist metoprolol and the β_2-adrenergic antagonist butoxamine was not particularly informative in that both inhibited binding and isoproterenol-stimulated enzyme activity similarly. Several studies [4, 11, 12] have shown that butoxamine is not completely specific for β_2-receptors.

The experiments with sulfhydryl compounds suggest that the β-adrenergic receptor in this tissue contains at least one disulfide moiety which is essential for (−)-[^{3}H]DHA binding. Specific binding was reduced drastically by incubating the membranes with 1,4- and 1,3-dithiols. The vicinal dithiol, 2,3-dimercaptopropanol, however, was much less effective and possessed a potency similar to the monosulfhydryl glutathione. The reduction reaction with dithiols normally occurs with formation of a mixed disulfide intermediate followed by formation of a stable cyclic dithiol and the reduced protein disulfide [13, 14]. 2,3-Dimercaptopropanol probably acts as

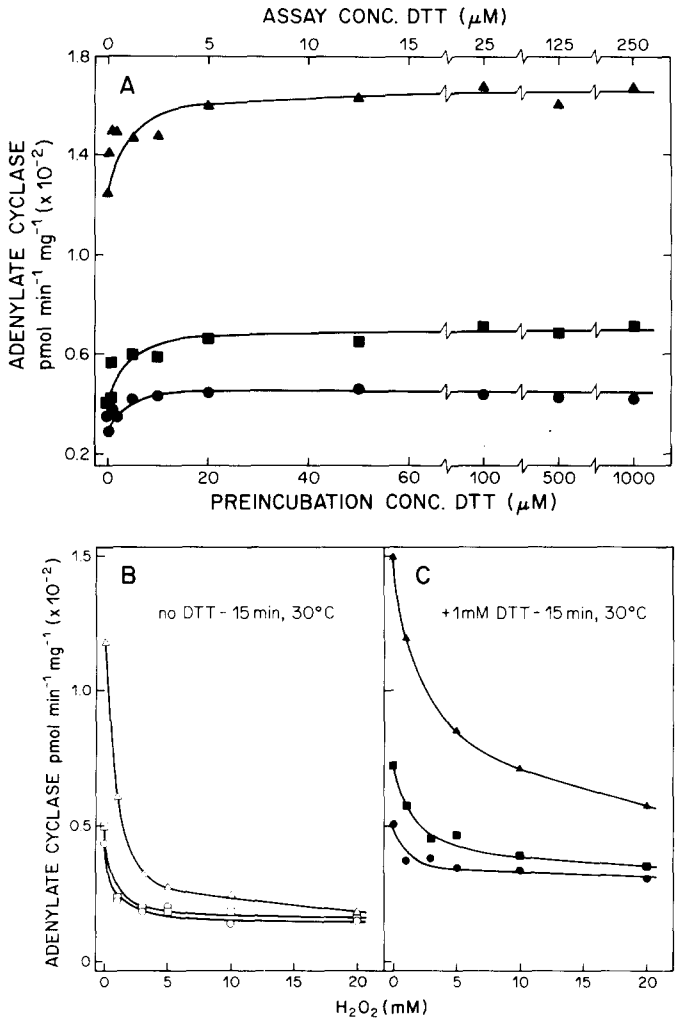

Fig. 6. Effect of DTT and H_2O_2 on adenylate cyclase. Panel A: membranes were incubated with various concentrations of DTT for 15 min at 30° and then assayed immediately for adenylate cyclase: (●) basal, (■) + 100 μM Gpp(NH)p, (▲) + 100 μM Gpp(NH)p + 10 μM (−)-isoproterenol. Panels B and C: membranes were added to the assay mixture containing all components except ATP and pyruvate kinase. H_2O_2 was added in various concentrations, and the tubes were incubated for 15 min at 4°. H_2O_2 was destroyed by the addition of 5 μg catalase (in 10 μl) to each tube and incubation for 10 min at 30°. To half the tubes DTT was added to a final concentration of 1 mM and incubation was continued for 15 min. Adenylate cyclase assay was initiated by the addition of [^{32}P]ATP solution containing pyruvate kinase. Panel B: membranes treated with H_2O_2. Panel C: membranes treated with H_2O_2 and subsequently with DTT. Key: (○, ●) basal; (□, ■) + Gpp(NH)p; and (△, ▲) + Gpp(NH)p + (−)-isoproterenol.

a monosulfhydryl because oxidation to a cyclic disulfide would require formation of a strained four-membered ring. The primary effect of DTT on binding was a decrease in the number of binding sites; in fact, binding could be reduced to zero with a 10 mM concentration of the dithiol. A small change in receptor affinity also possibly occurred. Competition for residual (−)-[^{3}H]DHA binding sites by (−)-isoproterenol and (−)-alprenolol in membranes treated with submaximal (1 mM) DTT concentrations was not different from that in untreated preparations. DTT-induced inactivation could be partially reversed by H_2O_2, and the receptors could be locked in the inactivated state by alkylation. The fact that complete reversal of inactivation could not be achieved with H_2O_2 might indicate the presence of sulfhydryl groups in the vicinity of the disulfide so that upon oxidation a certain amount of random disulfide formation could take place which would not restore functional receptors. Protection of (−)-[^{3}H]DHA binding sites from the detrimental effects of DTT was seen with both agonists and antagonists. Protection most likely occurred by receptor occupancy by the ligands, since it followed the potency order of a β_2-adrenergic receptor and displayed stereospecificity (Table 1). In this experiment, binding of (−)-[^{3}H]DHA after treatment with agonists was significantly lower than in untreated membranes.

This could be a reflection of incomplete removal of agonists during subsequent washing of the membranes. Most likely it reflects agonist-induced desensitization of the receptors as reported for several other systems [3, 15–18]. In all these respects, the β_2-adrenergic receptor of skeletal muscle displayed striking similarities to the β_1-receptor of turkey erythrocytes [1]. Vauquelin *et al.* [1] suggested that receptor protection by adrenergic ligands in these cells resulted from either a conformational change or a shielding of the disulfide bridge caused by receptor occupancy by ligands.

It appears that an essential disulfide moiety may not fill all the requirements for agonist binding to the β-adrenergic receptor. It has been reported in studies with turkey erythrocytes [19, 20] and S49 lymphoma cells [21] that low concentrations of the sulfhydryl reagent *N*-ethylmaleimide inactivates β-adrenergic receptors in the presence of agonists. The reagent in the presence of an antagonist had no effect. From recent similar findings in rat lung membranes, Heidenreich *et al.* [22] have suggested that agonist binding causes the hormone–receptor complex to associate with the regulatory component (which binds guanine nucleotide) resulting in either the reduction of a disulfide or exposure of an existing sulfhydryl. The free sulfhydryl would then be accessible to alkylation by *N*-ethylmaleimide.

The results from the experiments on the effect of DTT on adenylate cyclase in the muscle membranes agree with observations from other studies [23–25] and are consistent with the enzyme having a sulfhydryl group which is crucial for catalytic activity. In heart preparations, we have shown [25] that the essential group is a dithiol. Sensitivity to oxidation was not as great in these skeletal muscle membranes as in heart membranes [25] where activity was almost entirely lost by washing the preparations in the absence of DTT. The skeletal muscle enzyme, however, was highly sensitive to the oxidizing action of H_2O_2 and this was partially reversed by DTT. Thus, it can be said that the β_2-adrenergic receptor of skeletal muscle contains an essential disulfide moiety and is coupled to adenylate cyclase which contains at least one essential sulfhydryl. This could be of considerable significance for the *in vivo* regulation of the hormone-stimulated enzyme.

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