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Selective Parasympathectomy Increases the Quantity of Inhibitory Guanine Nucleotide-Binding Proteins in Canine Cardiac Ventricle

TIMOTHY D. HODGES, JOHN C. BAILEY,* JOHN W. FLEMING, and RICHARD J. KOVACS*

Department of Pharmacology and Toxicology (T.D.H., J.W.F.), Department of Medicine, and the Krannert Institute of Cardiology (J.C.B., R.J.K.), Indiana University School of Medicine, Indianapolis, Indiana 46223*

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SUMMARY

In mammalian heart, vagal stimulation or the direct application of acetylcholine produces profound direct effects on the electrophysiologic characteristics of atrial myocytes. At the tissue level, these effects are observed as shortening of atrial action potential duration. Despite anatomic, biochemical, and physiologic evidence for significant vagal input to the mammalian ventricle, similar direct effects of acetylcholine on the ventricular action potential have been difficult to demonstrate. Chronic denervation via cervical vagotomy is one method that has been shown to render previously unresponsive ventricular myocytes sensitive to acetylcholine, but the molecular mechanism has not been defined. In the experiments described, selective cardiac parasympathectomy was performed on mongrel dogs. Five to seven days after parasympathectomy, the dogs were sacrificed, electrophysiologic responses to acetylcholine were measured, and sarcolemmal vesicles were prepared. After parasympathectomy, ventricular myocytes were responsive to the effects of acetylcholine, manifested as shortening of the action potential duration. A quantitative and functional assessment of the transmembrane signalling mechanisms of the muscarinic receptor was carried out. After parasympathectomy, the density of muscarinic receptors in the sarcolemma was increased, compared with control ventricles. After parasympathectomy, ventricular sarcolemma displayed significant increases in both basal and oxotremorine-stimulated GTPase activity. ADP-ribosylation revealed significantly increased quantities of the pertussis toxin substrates G_i and G_o. The quantity of ADP ribose incorporated was correlated with the increased level of GTPase activity in control and oxotremorine-stimulated membranes. Quantitation of the α and $\beta\gamma$ subunits of the guanine nucleotide-binding proteins by immunoblot confirmed the increase in density of inhibitory guanine nucleotide-binding proteins following parasympathectomy. The results offer new insights into possible mechanisms of altered electrophysiologic responsiveness to acetylcholine following cardiac parasympathectomy.

After parasympathetic denervation of the heart, the electrophysiologic effects of parasympathetic agonists on ventricular tissue are altered (1). The action potential duration of ventricular myocardium, which normally exhibits no direct response to ACh, shortens following vagotomy. To date, the mechanism of this altered responsiveness remains unclear. The effects of ACh in cardiac ventricle are mediated through muscarinic receptors and, presumably, regulatory G proteins. Previous experiments in the same model of cardiac parasympathectomy have revealed an increase in the density of sarcolemmal muscarinic receptors, but increased receptor density may not be sufficient to explain the observed physiologic changes (2). The next logical step in determining the mechanism of the observed response is to quantitate and functionally assess the G proteins associated with muscarinic receptors in the heart.

Activation of muscarinic receptors in the ventricular myocardium results in GTPase activity and inhibition of adenylate cyclase (3–5). The G proteins responsible for these activities are G_i and G_o, which are substrates for covalent modification by pertussis toxin (6). Studies of animals treated *in vivo* with pertussis toxin have demonstrated the need for functional G proteins in the regulation of transmembrane signalling (7, 8). After parasympathetic decentralization, the observed alteration in electrophysiologic responsiveness could be explained by intensified transmembrane signalling via G proteins. Three possible situations might exist: increased activation of an un-

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ABBREVIATIONS: ACh, acetylcholine; G protein; guanine nucleotide-binding protein; G_i, the inhibitory guanine nucleotide-binding protein of adenylate cyclase; G_o, a GTP-binding protein that regulates muscarinic receptor affinity; $G_{i\alpha}$, the M_r 41,000 (approximately) GTP-binding subunit of G_i, which is ADP-ribosylated by pertussis toxin; $G_{o\alpha}$, the M_r 39,000 (approximately) GTP-binding subunit of G_o, which is ADP-ribosylated by pertussis toxin; App(NH)p, adenosine 5′-[β,γ-imido]triphosphate; TEMED, *N,N,N′,N′*-tetramethylethylendediamine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; G_s, the stimulatory guanine nucleotide-binding protein of adenylate cyclase.

changed density of functional G protein, increased density of functional G proteins, or both increased density and increased activation. Alternatively, the electrophysiologic results might be explained by direct activation of K^+ or Ca^{2+} channels in the sarcolemma via G proteins, as has been demonstrated in both atria and ventricles (9, 10). Thus, quantitation and functional assessment of G_i and G_o are crucial to further characterization of the mechanism of altered sensitivity to ACh following parasympathectomy.

Experimental Procedures

Materials

[γ-^{32}P]GTP (29 Ci/mmol), [*adenylate*-^{32}P]NAD (30 Ci/mmol), and Formula-963 liquid scintillation solution were obtained from New England Nuclear (Boston, MA). Low molecular weight standards for SDS-PAGE, bis-acrylamide, acrylamide, SDS, bromophenol blue, ammonium persulfate, dithiothreitol, and TEMED were purchased from Bio-Rad Laboratories (Richmond, CA). Ultra-pure sucrose was obtained from Schwartz-Mann (Cambridge, MA) and App(NH)p was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Alkaline Norit-A charcoal was obtained from Fisher Scientific (Fairlawn, NJ). Antibodies to α and $\beta\gamma$ subunits of G_i and G_o were a gift of A. Gilman, University of Texas, Southwestern Medical Center, Dallas, TX. All other ligands, nucleotides, buffers, and reagents were from the Sigma Chemical Co. (St. Louis, MO).

Methods

Parasympathectomy. Adult mongrel dogs were anesthetized with secobarbitol (30 mg/kg, intravenously) and ventilated. The method used to achieve selective parasympathectomy was described by Randall *et al.* (11). This procedure results in complete decentralization of the heart from parasympathetic influence while leaving sympathetic nerves intact. Electrocardiogram lead II was monitored throughout the surgery. To confirm completeness of the parasympathectomy, the cervical vagi were stimulated (20 Hz, 5 msec, 5V) to demonstrate lack of effects on SA or AV nodal function. Lack of vagal effects on these parameters correlates well with total cardiac parasympathectomy (11). The animals were allowed to recover for 5–7 days. At the time of membrane preparation, the animals were again anesthetized and the cervical vagi were stimulated to confirm selective parasympathectomy. In some animals, choline acetyltransferase activity was determined in atrial and ventricular homogenates to further demonstrate completeness of the parasympathectomy (data not shown).

Microelectrode studies. Electrophysiologic parameters were measured in normal ventricle and ventricle following parasympathectomy, using conventional microelectrode techniques. Preparations were stimulated at 1 Hz. Action potential duration was stable for 15 min before superfusion with ACh. Dose-response experiments with ACh were performed, with concentrations of the drug applied in a random order. Data were digitized and entered into an IBM PC computer for analysis of action potential parameters, using custom-designed software previously described (12).

Membrane preparation. Experiments used a highly purified and extensively characterized canine cardiac sarcolemma prepared from left ventricle, as described by Jones (13). The total ouabain-sensitive Na^+,K^+-ATPase activity of the purified sarcolemma was 70–90 μmol of P_i/hr/mg of protein. The effect of parasympathectomy on the ability to purify sarcolemmal membranes was determined by assessing recovery of membrane protein and Na^+,K^+-ATPase activity, as previously described (13). Parasympathectomy had no effect on the amount of protein recovered during membrane purification (5.5 ± 0.2 mg of sarcolemma/100 g of left ventricle versus 5.7 ± 0.5 mg/100 g in control) or on its content of the marker enzyme Na^+,K^+-ATPase (74 ± 6 μmol of P_i/mg of sarcolemma versus 72 ± 6 μmol of P_i/mg of sarcolemma in controls). These data establish that subsequent quantitative differences in the measured activities of sarcolemmal proteins are not attributable to differences in membrane purification.

Muscarinic receptor binding. Muscarinic receptors were quantitated using the antagonist ligand [^{3}H]quinuclidinyl benzilate. Saturating amounts of [^{3}H]quinuclidinyl benzilate (5.5 nM) were displaced by increasing concentrations of the agonist oxotremorine in a buffer that contained 10 mM $MgCl_2$, 0.1 mM ascorbic acid, and 40 mM Tris·HCl (pH 7.5). Binding was allowed to proceed to equilibrium for 10 min at 37°, before filtration on Whatman GF/C filters.

GTPase Assay. GTP hydrolysis was determined by measuring the release of $^{32}P_i$ from [γ-^{32}]GTP (14). Standard assay conditions contained the following final concentrations of reagents: 40 mM Tris·HCl, 0.25 μM (3000 cpm/pmol) [γ-^{32}]GTP, 5 mM creatinine phosphate, 5 units/ml creatinine phosphokinase, 1 mM ATP, 1 mM APP(NH)p, 10 mM $MgCl_2$, 1 mM EDTA, and 6 μg of membrane protein. The concentration of oxotremorine was 10 μM. Specific GTPase activity was determined by subtracting nonspecific P_i release from mean total activities. Results are expressed as the mean specific GTPase activity ± standard deviation of individual experiments or as mean ± standard error of multiple experiments.

^{32}P-Ribosylation of $G_{i\alpha}$ and $G_{o\alpha}$. Covalent labeling of the α subunits of the pertussis toxin substrates was performed by a modification of the method of Bokoch *et al.* (15). Sarcolemmal samples were solubilized during a 60-min preincubation in an equal volume of TED (20 mM Tris·HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol) containing 1% cholate. The solubilized samples (1–3 mg/ml) were then diluted to a final concentration of 0.15 mg/ml in TED containing 0.05% Lubrol. The ADP-ribosylation reaction was initiated by adding 10 μl of the dilute protein solution to 26 μl of reaction buffer. The final concentrations of the reagents in the reaction mixture were 100 mM Tris·HCl, pH 8.0, 1 mM ATP, 100 μM GTP, 2.5 mM $MgCl_2$, 10 mM thymidine, 1 mM EDTA, 500 μM L-α-dimyristoyl phosphatidylcholine, 1 μM [^{32}P] NAD (30,000–40,000 cpm/pmol), and 3 μg/ml islet-activating protein (preactivated with 20 mM dithiothreitol for 15 min at 37°). The reactions proceeded for 60 min at 30°, approximately twice the time required for full labeling (data not shown). The reactions were terminated by the addition of an equal volume of solubilization buffer [0.125 M Tris, pH 6.8, 2.5% (v/v) SDS, 20% (v/v) glycerol, 0.1% (v/v) bromophenol blue, 0.5% (v/v) 2-mercaptoethanol]. Samples were then boiled for 5 min.

SDS-PAGE. After incubation in the absence or presence of pertussis toxin, membranes were analyzed by SDS-PAGE as described by Laemmli (16). Gels were cast as discontinuous systems (3.7% stacking gel, 12% resolving gel), at a thickness of 0.75 mm. Samples containing 1–1.5 μg of protein were loaded into each well. The gels were run at 40 mA/gel for 75 min after the bromophenol blue dye front exited from the bottom of the gel. To locate low molecular weight standards, the gels were stained with Coomassie blue and then destained. The gels were then dried in the absence of heat using BioFrame GelWrap (Bio Design Inc., Carmel, NY). To visualize extracted membrane proteins, the gels were stained with alkaline silver, according to the method of Wray *et al.* (17). To evaluate the amount of [^{32}P]ADP-ribose incorporated into the regulatory proteins G_i and G_o, individual bands were located by autoradiography, cut from the lanes, and counted in 10 ml of scintillation fluid. Control lanes were run in the absence of pertussis toxin, and total counts incorporated were calculated by subtracting the control lane counts from counts incorporated in the presence of pertussis toxin. Values for ADP-ribosylation are expressed as pmol of [^{32}P]ADP-ribose incorporated/mg of protein.

Immunoblots. Gel electrophoresis, transfer of proteins to nitrocellulose, and processing of blots were carried out by modifications of procedures previously described (18). Samples were pretreated with *N*-ethylamaleimide for electrophoresis for 11% polyacrylamide gels, as described by Sternweis and Robishaw (19). The separated proteins were then transferred from the gel to nitrocellulose paper (Schleicher and Schull, Keene, NH) or Immobilon PYDF (Millipore, Bedford, MA) by use of an electrophoretic transfer apparatus (Hoeffer Scientific, San

Francisco, CA) operated at a constant voltage of 30 V for 16 hr. After transfer, the membrane substrate was incubated in 50 mM Tris, pH 8.0, containing 5% (w/v) bovine serum albumin, 0.2% Nonidet P-40, 0.02% sodium azide, 2 mM $CaCl_2$, and 80 mM NaCl (buffer A), for 1 hr. A 1-hr incubation with antiserum to either the subunit or common subunit (U 49 and J881, respectively; gifts of A. Gilman) was followed by three 15-min washings in buffer A. Antibody binding was detected by incubation of the blot with ^{125}I-labeled goat anti-rabbit IgG F(ab')$_2$ (New England Nuclear) at 2.5×10^5 cpm/ml in buffer A. The blot was then washed three times for 15 min, rinsed twice (with buffer A minus Nonidet P-40 and bovine serum albumin), and washed twice with this solution for 10 min. Blots were allowed to air dry and were subjected to autoradiography for 15 hr with Dupont Cronex film and an intensifying screen at −70°. Regions of the blots that correspond to bands on the autoradiograms were excised and the amount of radioactivity was quantified with a γ-counter. Transfers of sarcolemmal proteins under these immunoblot conditions were quantitative between 5 and 75 μg of control sarcolemmal protein. The amount of radiolabeled second antibody associated with each antigen band varied linearly with the amount of membrane protein over a range of 0–75 μg (data not shown). A total of 25–30 μg of sarcolemma were analyzed for each control ventricle and ventricle following parasympathectomy.

Miscellaneous. Protein estimation of membrane preparations was by the method of Lowry *et al.* (20). The small amount of protein in each ADP-ribosylation extract was quantitated by the Amido black method (21). Bovine serum albumin was used as the standard in both methods. Pertussis toxin was prepared by growing *Bordetella pertussis* in modified Stainer-Sholte (22) medium, as described by Hewlett *et al.* (23). Statistical evaluation of differences between means was performed by Student's *t* test. Product-moment correlation coefficients were used to determine significance of correlation in bivariate scattergrams (24).

Results

Action potential duration response to ACh. Following parasympathectomy, ACh produced a shortening of ventricular myocyte action potential duration, in a concentration-dependent manner (Fig. 1). Fig. 1, *top*, is a representative experiment from normally innervated dog ventricle. As increasing amounts of ACh are added to the superfusate, no change in action potential duration is observed. Fig. 1, *bottom*, is a representative experiment from a ventricle following parasympathectomy. Parasympathectomy did not alter action potential duration in the absence of ACh. However, superfusion with increasing concentrations of ACh produce shortening of action potential duration at both 50 and 90% of repolarization (six experiments; $p < 0.01$). These effects were reversed by the addition of 10^{-6} M atropine to the superfusate. The effect of ACh on action potential duration was not dependent on the presence of catecholamines. Addition of propranolol (10^{-6} M) to the superfusate did not alter the response to ACh in either normally innervated ventricle or ventricle following parasympathectomy. These data are comparable to previous experiments in the cat (1) and imply that this effect of ACh is not species specific.

Muscarinic receptor binding. Total muscarinic receptor density in control ventricles (five experiments) was 2.32 ± 0.23 pmol/mg of membrane protein. In membranes from ventricles after parasympathectomy (five experiments), receptor density was 4.61 ± 0.46 pmol/mg of protein. This 2-fold increase in density was statistically significant ($p < 0.05$).

Basal and hormone-stimulated GTPase activities. Basal and oxotremorine-stimulated GTPase activity were determined in five dogs after parasympathectomy and in seven normally innervated dogs. GTPase activity was presumed to be due to activity of inhibitory G proteins, because G_s produces an insignificant contribution to GTPase activity in canine sarcolemma (3). Both basal and oxotremorine-stimulated GTPase activities were significantly elevated in the animals after parasympathectomy ($p < 0.001$). Basal GTPase activity was 15.14 ± 0.92 units/mg in normal ventricles, and increased to 27.36 ± 2.88 pmol of P_i/min/mg in ventricles after parasympathectomy. Maximal oxotremorine (10 μM) concentrations stimulated the GTPase activity to 24.22 ± 1.29 pmol of P_i/min/mg in normal and to 42.86 ± 4.10 pmol of P_i/min/mg in operated animals. Parasympathectomy increased both basal GTPase activity and hormone-stimulated activity by about 80% (79.3 ± 4.8 and 77.0 ± 7.4, respectively). The ability of muscarinic agonists to stimulate the high affinity, low K_m, GTPase of canine cardiac sarcolemma was similar in normal ventricles (60.6 ± 2.9%) and ventricles after parasympathectomy (57.5 ± 3.9%).

[^{32}P]ADP-ribosylation of G_i and G_o. [^{32}P]NAD/pertussis toxin labeling was used to quantitate G_i and G_o in sarcolemma from the two groups of dogs. ADP-ribosylation of intact sarcolemmal vesicles was initially performed as reported previously (7). This method involved the labeling of intact vesicles, followed by centrifugation and resuspension of the protein sample in dissociation buffer. Fig. 2A is a scattergram of GTPase activity versus ADP-ribosylation of intact vesicles. Incorporation of [^{32}P]ADP ribose into control membranes was 0.143 ± 0.028 pmol/mg of protein (four experiments), whereas that of the membranes from ventricles after parasympathec-

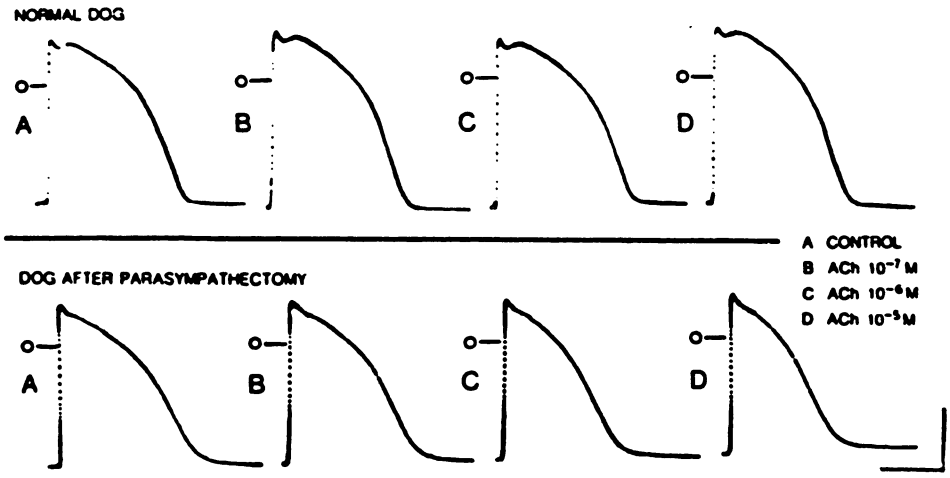

Fig. 1. Ventricular action potentials in multicellular preparations of normally innervated ventricle and ventricle following parasympathectomy. Action potentials were collected as described in Experimental Procedures. The *A panel* of each *row* is recorded in the absence of ACh, whereas *panels B, C,* and *D* represent increasing concentrations of ACh. *Calibration markings* are shown in the *lower right corner.* The *horizontal line* corresponds to 100 msec, whereas the *vertical line* represents 40 mV. ACh produced no change in action potential amplitude, resting membrane potential, or upstroke velocity in either preparation. ACh produced significant shortening of the action potential duration in ventricle following parasympathectomy.

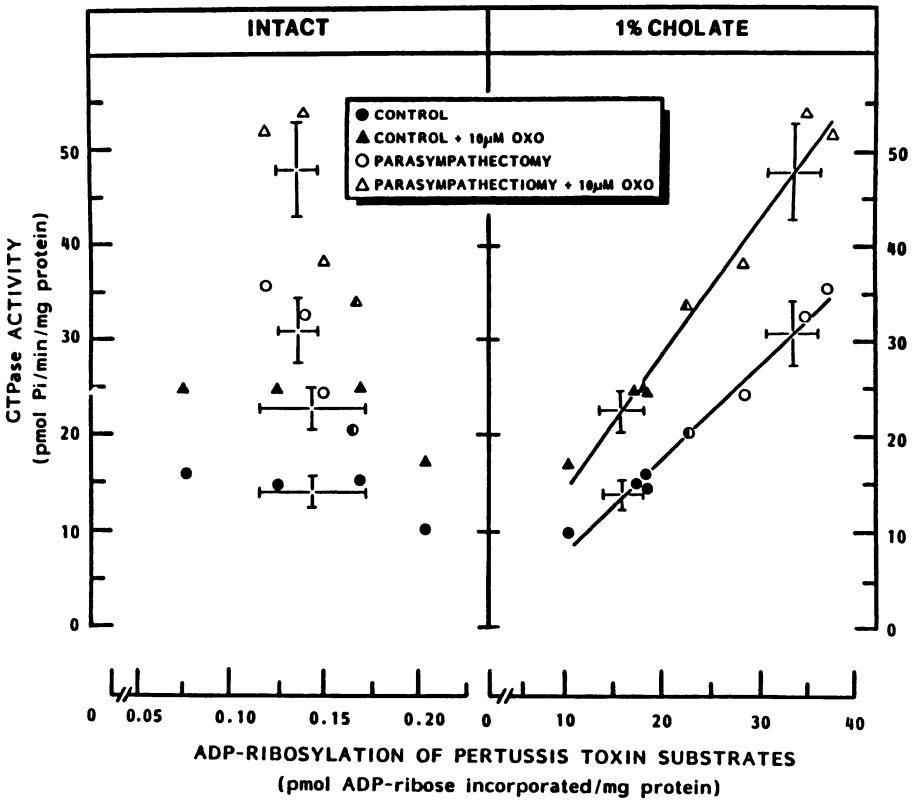

Fig. 2. Correlation of basal and oxotremorine-stimulated specific GTPase activity versus ADP-ribosylation of pertussis toxin substrates. Basal and oxotremorine-stimulated GTPase activities were determined in membranes purified from normally innervated ventricle and ventricle following parasympathectomy, as described in Experimental Procedures. ADP-ribosylation of G_i and G_o was carried out as described in Experimental Procedures. Correlation was determined by calculating the product moment correlation coefficient. Significance of the correlation was assessed by performing a *t* test. The intersection of *horizontal* and *vertical lines* represents the mean ± standard error for each of the four parameters. ◐ and ◭ represent an animal that was an "incomplete parasympathectomy" (see Results). N = 4, normally innervated; n = 3, following parasympathectomy.

tomy was 0.136 ± 0.009 pmol/mg (three experiments). Correlation between GTPase activity and ADP-ribosylation was not evident in either the basal (r = −0.307) or oxotremorine-stimulated (r = −0.273) conditions. ADP-ribosylation was then performed on the same sarcolemmal samples after extraction of membranes by cholate (see Experimental Procedures). Autoradiography of SDS-PAGE analysis revealed covalent incorporation of [^{32}P]ADP-ribose into two protein bands, of M_r 41,000 and 39,000. Ribosylation was specific, and no other proteins were significantly labeled. Incorporation of radioactive label into these two bands was highly correlated with the basal GTPase activity of the same sarcolemmal preparation. Fig. 2B shows the significant association between GTP hydrolysis and ADP-ribosylation of G_i and G_o (correlation coefficient = 0.993; $p < 0.001$) for both normally innervated ventricles and ventricles after parasympathectomy. These results also indicate that ventricles after parasympathectomy have significantly more labeled G_i and G_o (33.85 ± 2.64 pmol/mg of protein; three experiments) than control ventricles (16.19 ± 1.91 pmol/mg of protein; four experiments) ($p < 0.02$). Correlation of oxotremorine-stimulated ADP-ribosylation of pertussis toxin substrates was also highly significant (correlation coefficient = 0.985; $p < 0.001$). It is of interest to note that one animal exhibited "incomplete parasympathectomy," which was demonstrated by observing 2:1 AV block in response to vagal stimulation at the time of sacrifice, although parasympathectomy appeared complete at the time of surgery. The ribosylation values for this animal were intermediate between those of the normally innervated group and the group following parasympathectomy (Fig. 2).

Immunoblotting. U-49 antiserum reacted with a single protein band on nitrocellulose (Fig. 3B), of M_r 36,000. No other proteins were labeled. Quantitation of ^{125}I incorporation re-

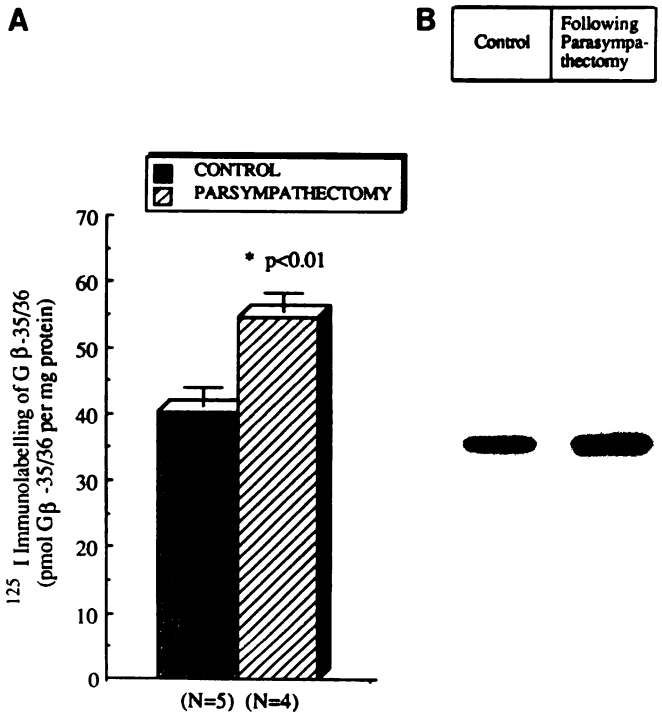

Fig. 3. The effect of parasympathectomy on the density of β subunits. Purified sarcolemmal vesicles from control animals and from animals following parasympathectomy were incubated with U49 antisera as described in Experimental Procedures and were labeled with ^{125}I-goat anti-rabbit IgG F(ab')$_2$ fragment. *Left*, the *bars* represent the mean ± standard error expressed as pmol of β subunit/mg of protein. Differences were analyzed by *t* test, and significance is designated by an *asterisk*. *Right*, a representative autoradiogram showing the increase in immunolabeling following parasympathectomy.

vealed a significantly increased density of the β subunit in sarcolemma following parasympathectomy (Fig. 3A). J-881 antiserum reacted with four protein bands on PVDF membranes (Fig. 4B). Quantitation of ^{125}I incorporation in the 41- and 39-kDa bands of these blots also revealed a significant increase in the density of $G_{i\alpha}$ and $G_{o\alpha}$ in sarcolemma after parasympathectomy (Fig. 4A).

Discussion

Chronic selective cardiac parasympathectomy of the canine heart produces important changes in the electrophysiologic response to ACh. The ventricle is converted from an organ that displays only indirect responsiveness to ACh [ACh antagonizes the effect of simultaneous β adrenergic stimulation (25)] to an organ that exhibits a direct response to ACh. If the role of the vagus can be viewed as primarily cardioprotective (slowing the heart rate, slowing conduction, and protecting against the adverse effects of catecholamine stimulation), processes that accentuate the parasympathetic response may be of potential therapeutic importance.

The model of selective parasympathectomy may also be important in the study of processes that influence muscarinic receptor density. Changes in autonomic receptor density have been observed in several disease states, including thyroid disease, heart failure, and diabetes. Most studies have emphasized the adrenergic receptor population, and results of muscarinic receptor studies have been inconclusive. Parasympathectomy offers a model system to study the correlation of changes in muscarinic receptor density with physiologically relevant responses as well as the alterations in the receptor-effector coupling system demonstrated in these experiments.

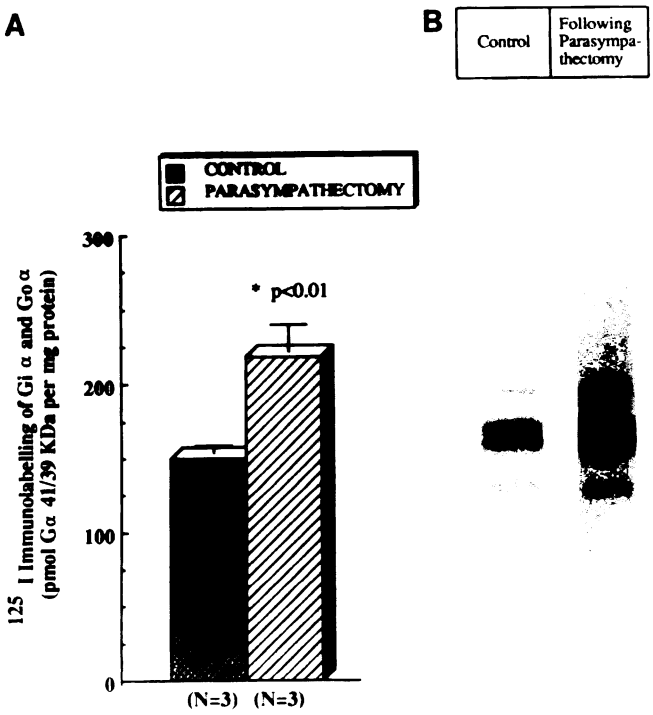

Fig. 4. The effect of parasympathectomy on the density of $G_{i\alpha}$ and $G_{o\alpha}$ subunits. Purified sarcolemmal vesicles from control animals and from animals following parasympathectomy were incubated with J881 antisera as described in Experimental Procedures and were labeled with ^{125}I-goat anti-rabbit IgG F(ab')$_2$ fragment. a, The *bars* represent the mean ± standard error of combined density of $G_{i\alpha}$ and $G_{o\alpha}$ expressed as picomol of G_i and G_o subunit/mg of protein. Differences were analyzed by *t* test, and significance is designated by an *asterisk*. b, A representative autoradiogram showing the increase in immunolabeling following parasympathectomy.

Selective parasympathectomy of the heart produced quantitative and functionally significant increases in the inhibitory G proteins in ventricular sarcolemma. Increased ability of membranes to hydrolyze GTP directly correlated with the quantity of G proteins detected by ADP-ribosylation and paralleled the increases revealed by quantitative immunoblotting using specific antisera. The observed increase in the rate of GTPase activity could be postulated to be due to either 1) an increased rate of hydrolysis by a similar density of G protein (presumably due to increased G protein/receptor interaction), 2) an increased density of G protein hydrolyzing at the same rate, or (3) a combination of increased density and increased rate. Under conditions that optimize the rate of GTP hydrolysis (maximal oxotremorine stimulation), parasympathectomy still produced a marked increase in GTPase activity. These experiments would indicate that the most likely explanation for the increased GTPase activity is an increase in density.

Solubilization of sarcolemma before ADP-ribosylation was required to reveal the increased density of G proteins. Labeling of intact sarcolemmal vesicles with pertussis toxin results in a constant low level of incorporation. Presumably only small amounts of G_i and G_o are exposed in intact vesicles. The region of modification of G_i and G_o by pertussis toxin is near the COOH-terminus (26), the portion of the protein involved in receptor/G protein interaction (27). This may explain why these molecules need to be solubilized from the membrane in order to be more completely labeled. Our data indicate that GTPase activity determination and ADP-ribosylation of cholate extracts are an accurate means of assessing relative densities of inhibitory G protein in sarcolemmal membranes (Fig. 2B). These data correlate with direct quantitation by immunoblotting. Extraction of membranes before ribosylation may reveal significant differences in G protein density in other systems, which have previously gone undetected.

Although receptor density may change in response to disease states, alterations of G protein density in response to disease or physiologic stimuli are not as well characterized. Studies on canine tissue have indicated that, in response to heart failure, G_s density is reduced by 50%, whereas densities of G_i and G_o remain unchanged (28). In contrast, studies of the failing human heart have shown a 36% increase in G_i (29). These studies used pertussis toxin-catalyzed ADP-ribosylation as the principal means of quantitating cardiac G_i and G_o. Selective parasympathectomy is the first non-heart failure model to demonstrate an increase in the density of pertussis toxin substrates following *in vivo* experimental manipulation.

In the current investigation, we have not attempted to differentiate between ribosylation of G_i and G_o. Quantiative immunoblotting of G_i and G_o, however, demonstrated that, whereas both G_i and G_o levels increased after parasympathectomy, G_o levels increased proportionally more than those of G_i (data not shown). There is ample support for the notion that G_o, in addition to G_i, is coupled to the muscarinic receptor (30). Unlike the well characterized attenuation of adenylate cyclase associated with G_i, the function of G_o is not known, but it may be linked with phosphatidylinositol turnover and Ca^{2+} mobilization (31). Recently, direct effects of G proteins on cardiac K^+

(9) and Ca^{2+} (10) channel activity have been demonstrated. Selective parasympathectomy offers a physiologically relevant model to test the hypothesis that increasing the density of G proteins in sarcolemma could lead to direct alteration in sarcolemmal ion flux. Investigation of the possibility that one of these proteins is selectively responsible for the electrophysiologic changes associated with cardiac parasympathectomy will be the subject of future experiments.

Selective cardiac parasympathectomy produces alteration of the muscarinic receptor-effector system that may be responsible for important physiologic responses. The model may represent a reproducible way of altering functional inhibitory G protein density in ventricular membranes and, as such, may contribute to an understanding of the role of these proteins in relevant physiologic processes in both the normal and diseased heart.

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Send reprint requests to: Richard J. Kovacs, M.D., Krannert Institute of Cardiology, Indiana University School of Medicine, 1001 West 10th St., Indianapolis, IN 46202.