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GENETIC INFLUENCES ON AGONIST BINDING TO CARDIAC β-RECEPTORS

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Abstract—Regulation of β-adrenoceptor–agonist function in the Maudsley Reactive (MR/Har) and the Maudsley Non-Reactive (MNRA/Har) rat strains was assessed by comparison of isoproterenol competition for [^{125}I]iodocyanopindolol (ICYP) binding sites in crude left ventricular homogenate preparations. Non-linear, least-squares analysis of isoproterenol competition for ICYP binding in the absence of guanine nucleotide revealed different proportions of high- and low-affinity receptors in the two strains; MR/Har rats (59 ± 3.3%) had a significantly greater proportion of receptors in the high-affinity state than the MNRA/Har rats (41 ± 4.5%). Addition of the non-hydrolyzable guanine nucleotide analog guanylylimidodiphosphate (Gpp(NH)p) converted receptors to the low-affinity state. Analysis of Gpp(NH)p concentration–response curves in left ventricular homogenates of the two strains revealed that the MR/Har strain had a significantly ($P < 0.02$) lower EC_{50} for guanyl nucleotide inhibition of isoproterenol competition for ICYP binding than the MNRA/Har. Confirming previous experimental results, a significantly ($P < 0.04$) greater density of ventricular β-receptors was found in MR/Har rats (13.16 ± 0.92 fmol/mg protein) than in MNRA/Har rats (10.81 ± 0.63 fmol/mg protein). Left ventricular catecholamine levels were found to be correlated inversely with β-adrenoceptor density in the two strains; norepinephrine (NE) and epinephrine (EPI) concentrations (ng/mg protein) in left ventricle were 12.19 ± 0.94 for NE and 0.165 ± 0.038 for EPI in MNRA/Har, and 8.73 ± 0.95 and 0.018 ± 0.018, respectively, in MR/Har. All other parameters of agonist interactions with the cardiac β-adrenoceptor for the MR/Har and MNRA/Har rat strains were similar [the IC_{50} for displacement of ICYP binding by isoproterenol, the accompanying Hill coefficients in the Gpp(NH)p present and absent condition, the K_d of the high- and low-affinity states in the absence of Gpp(NH)p, and the K_d of the uniform low-affinity state in the presence of Gpp(NH)p]. We hypothesize that the strain-dependent differences in high-affinity state formation reported here may account for some of the *in vivo* differences in cardiovascular function previously demonstrated in the Maudsley rats.

The Maudsley strains differ in behavioral and physiological responses to a wide range of stressful stimuli [1, 2] and display differences in open-field defecation (OFD), a psychophysiological index of response to mild stress (Maudsley Reactive, high defecating; Maudsley Non-Reactive, low defecating). Using the Harrington derivation of the Maudsley strains (the MR/Har and MNRA/Har), the MR/Har rats were found to have markedly higher systolic and diastolic blood pressure and heart rate, coupled with lower tissue and plasma norepinephrine than MNRA/Har rats [3]. More recently, we demonstrated genetic variation in cardiac β-adrenoceptor characteristics in these strains: MR/Har rats have a greater density of cardiac β-adrenoceptors than the MNRA/Har rats [4].

The constellation of differences described above provides a useful model for studying genetic modulation of the biochemical mechanisms underlying cardiac β-receptor function. However, simple measurements of total receptor density as detected by Scatchard analysis of antagonist radioligand binding (described above [4]), or IC_{50} values determined from competition binding curves, do not adequately assess subtle yet important features of receptor regulation. Use of non-linear, least-squares computer modeling techniques in the analysis of radioligand binding studies has elucidated important interactions between agonist and the β-receptor [5–7]. The goal of the present study was to explore genetic influences on cardiac β-adrenoceptor–agonist interactions in the Maudsley rats using these modeling techniques.

MATERIALS AND METHODS

Experimental animals

Adult male rats (age 90–120 days) of the MR/Har and MNRA/Har strains were provided from the colony maintained by Dr. Gordon M. Harrington (Department of Psychology, University of Northern Iowa, Cedar Falls, IA). The Harrington colonies of the original Maudsley strains were derived from their British ancestors at the twenty-fifth generation of inbreeding, and have been maintained by brother/sister mating for approximately forty additional generations [2].

Experimental animals were maintained in the institutional vivarium on a 15-hr light/9-hr dark cycle with food and water available *ad lib*. Sacrifice times were approximately half-way through the light phase of the diurnal cycle.

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Homogenate preparation

All animals were killed by decapitation. The thoracic cavity was opened, and the heart was removed and placed on ice. The atria and right ventricular free wall were dissected, and the left ventricle was either placed in ice-cold saline for immediate use, or stored frozen at −70° in a Revco freezer for future use. Fresh and frozen tissues behaved similarly in binding experiments.

Tissue was cut into small pieces and homogenized in 10 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 10 mM $MgCl_2$, 1 mM guanosine triphosphate (GTP) (see below), and 50 μM phenylmethylsulfonyl fluoride (PMSF; a protease inhibitor). Homogenizations were performed with a Tekmar SDT-100 EN homogenizer at setting 5 for 30 sec. Suspensions were diluted with another 20 ml of homogenization buffer, and centrifuged at 48,000 *g* for 10 min. Tissue was then homogenized (at setting 3 for 10 sec), centrifuged as before, and resuspended four times, before a final resuspension in 20% (w/v) 50 mM Tris–HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 50 μM PMSF for assays. The multiple washings and resuspensions were carried out to remove exogenously administered GTP (see below). The first two resuspensions were passed through a tea-sieve to remove large pieces of connective tissue. Protein determinations were performed by the method of Lowry *et al.* [8], using bovine serum albumin as standard.

Exogenous GTP was added routinely to our homogenate preparation to remove endogenous catecholamines, because their presence can complicate interpretation of radioligand binding assays by the formation of high-affinity, slowly dissociable complexes, which mask a significant proportion of the β-adrenoceptor population [9]. We recently confirmed these findings when initial studies demonstrated that addition of GTP during homogenization significantly increased the B_{max} from 12.27 ± 1.11 to 13.59 ± 1.65 and 9.43 ± 1.08 to 11.29 ± 1.25 in the MR/Har and MNRA/Har, respectively, as determined by two-way analysis of variance, with a post-hoc Newman–Keuls test ($P < 0.02$, $N = 6$).

Rate of disappearance of [^{3}H]*guanosine triphosphate*

The course of removal of exogenously administered guanine nucleotide and its metabolites from left ventricular homogenate preparations was studied to demonstrate that it was not present in sufficient concentration to interfere with our binding assay. [^{3}H]Guanosine triphosphate ([^{3}H]GTP) from Amersham (sp. act. = 2.22×10^{16} dpm/mol) was added to a 1 mM GTP solution prior to homogenization, to give a final specific activity of 2.22×10^{12} dpm/mol. Left ventricular tissue was then homogenized in buffer containing the [^{3}H]GTP, and aliquots of the tissue resuspension were then taken after each successive homogenization.

Radioactivity present in each pair of aliquots taken was quantitated by liquid scintillation counting (corrected for quench) and used to approximate the concentration of GTP and its metabolites present both initially and after each successive rinse. Disappearance of radioactivity occurred rapidly and approached asymptote by the fifth rinse, with no difference in the rate of disappearance between the two strains. Addition of exogenous GTP followed by only three rinses resulted in 36% (36.3 ± 1.76, N = 3) of receptors in the high-affinity state (%RH) in Sprague–Dawley left ventricular homogenates; this value was increased significantly ($P < 0.005$, 12 d.f., Student's *t*-test) to 55% (55.3 ± 2.8, N = 10) upon addition of two more rinses of the homogenate preparations, illustrating the importance of five rinses.

β-Adrenergic receptor binding assay

β-Adrenergic receptor binding studies in left ventricular membranes were conducted using [^{125}I]iodocyanopindolol (ICYP) from New England Nuclear. Isoproterenol competition studies for ICYP binding were performed both in the presence and absence of a 100 μM concentration of the stable guanine nucleotide analog, 3′,5′-guanylylimidodiphosphate [Gpp(NH)p]. All samples were assayed in triplicate, with ICYP used at a final concentration of ≈ 25 pM.

Fifteen point isoproterenol competition curves were developed in which competing ligand [(−)isoproterenol], Gpp(NH)p, and ICYP were added to each appropriate tube, and the volume was adjusted to 200 μl by addition of Tris–HCl buffer. Reaction was begun by addition of 50 μl of left ventricular homogenate. The density of β-adrenoceptor binding sites (B_{max}) in the two strains was estimated from five-point Scatchard curves ranging from 6.25 to 100 pM ICYP. Total binding and non-specific binding were determined at each concentration in a final volume of 250 μl.

All tubes were incubated for 1 hr at 37°, for both competition binding experiments and Scatchard curves. Incubations were terminated by addition of 4 ml of ice-cold buffer to each tube, and bound and free drug were separated by vacuum filtration through Whatman GF/C glass fiber filters. Each sample tube was washed with three additional 4-ml aliquots of buffer. Filters were placed in polypropylene test tubes for quantitation of bound radioactivity. Specific binding was determined as the difference between ICYP binding in the presence and absence of 10 μM alprenolol.

Guanyl nucleotide concentration–response curves

Concentration–response curves were constructed to examine the effect of increasing concentrations of guanine nucleotide on isoproterenol displacement of ICYP binding in MR/Har and MNRA/Har left ventricular homogenates. For these experiments, left ventricular homogenate preparations from the MR/Har and MNRA/Har were incubated with Tris–HCl assay buffer, 500 nM isoproterenol, 25 pM ICYP and increasing concentrations of Gpp(NH)p ranging from 100 nM to 100 μM, in a final volume of 250 μl.

Tissue catecholamine determinations

Tissue extraction. The method of Sundberg *et al.* [10] was used for extraction of left ventricular catecholamines. Briefly, left ventricular tissue was homogenized in 0.5 to 1 ml of 0.02 N acetic acid in 98% methanol containing 2.5 ng of the internal

amine standard, dihydroxybenzylamine (DHBA). Aliquots of the supernatant fraction (100–500 μl) were adjusted to pH 8.6 with 0.7 ml of 0.5 M Tris-HCl buffer. Activated alumina (15 mg) was added to each aliquot to extract the biogenic amines. The alumina was then washed twice with distilled H_2O prior to eluting the catechols with 50 μl of 0.1 N HCl. Pellets were saved for protein measurement by the method of Lowry *et al.* [8].

Two different tissue conditions were assessed for catecholamine levels in MR/Har and MNRA/Har left ventricles as follows:

Original—denotes section of tissue (≈ 50 mg) cut from left ventricle, homogenized once in ice-cold methanol–acetic acid, centrifuged, and subjected to immediate catecholamine analysis. These samples provide the level of catecholamines initially present in the left ventricle of the two strains.

Pellet—denotes section of tissue (5–20 mg) homogenized in ice-cold Tris–HCl assay buffer in the presence of exogenous GTP and rinsed five times by centrifugation and resuspension. Final pellets were stored in 0.5 ml methanol–acetic acid at −70° until resuspension by sonication and catecholamine analysis. These samples provide the amount of residual endogenous catecholamine present in left ventricular homogenates used for binding assays.

Measurement of catecholamine content. Reverse-phase high-performance liquid chromatography with electrochemical detection was used to measure left ventricular catecholamine levels [10]. The mobile phase, containing 0.1 M NaH_2PO_4 (7.9 g/liter), 1.0 mM EDTA (372 mg/liter), and 50 mM heptane sulfonate (120 mg/liter) in deionized water, was vacuum filtered through a 0.45 μM pore size nylon-66 filter prior to use. A 50-μl aliquot of extracted sample was injected onto a C_{18} μ-Bondapak (Waters) reverse-phase column with a guard column. An amperometric detector (Bioanalytical Systems) with a Glassy Carbon, TL-8A Electrode with RE-3 reference electrode was used to quantitate catecholamine levels, with output recorded on a Hewlett–Packard 3390A integrator. Measurement of all catecholamines was adjusted for recovery of the internal amine standard, DHBA.

Data analysis

Radioligand binding data were analyzed by use of a weighted non-linear, least-squares curve-fitting technique described elsewhere [11]. The computer program used was KINETIC, EBDA, LIGAND, and LOWRY (Elsevier Biosoft). The non-corrected value of specific activity for ICYP was used [12]. Isoproterenol competition data were analyzed for the model of best fit. Initial estimates of IC_{50} values and receptor density from EBDA were provided to the LIGAND program, and iteratively refined until the weighted sum of the squares was minimized. Model hypotheses were verified by an approximate F-test to determine if there was a significant reduction in the weighted sum of squares given the change in the model (e.g. one site vs two sites). In no case was a two-site model accepted over a one-site fit unless it provided a significantly improved fit.

Analysis of the guanyl nucleotide sensitivity curve was performed using a technique described elsewhere [13]. The computer program used was Dose–Effect Analysis with Microcomputers (Elsevier Biosoft). Briefly, Michaelis–Menten kinetics were used to obtain K_m (Michaelis constant; half-saturation concentration) and V_{max} (maximum initial reaction rate) values. The percent of V_{max} at each Gpp(NH)p concentration was then calculated and utilized in Multiple Drug–Effect Analysis to determine the EC_{50} value.

For comparison of agonist binding parameters, two-way analysis of variance for repeated measures was utilized, with the Newman–Keuls test employed for post-hoc analysis of pairwise differences. However, for NE levels in left ventricle, a one-way analysis of variance with a post-hoc Newman–Keuls test was utilized. Because of the large number of left ventricular preparations with undetectable EPI levels in the MR/Har (i.e. a value of zero), the Mann–Whitney U test was used to evaluate strain influences on original EPI levels in left ventricular homogenates.

RESULTS

Receptor density

Scatchard analysis of radiolabeled antagonist binding confirmed previous findings in whole heart [4], and revealed that the MR/Har also has a significantly greater number of β-adrenoceptor binding sites than the MNRA/Har in left ventricle (MR/Har B_{max} = 13.16 fmol/mg protein, MNRA/Har B_{max} = 10.81 fmol/mg protein; $P < 0.04$, 35 d.f., Student's *t*-test).

Agonist competition studies

We also found that isoproterenol displaced ICYP from rat cardiac membranes in a predictable manner in both strains. A typical example of experimental data is shown in Fig. 1, which is a computer-generated curve modeled by the ligand program for an MR/Har rat ventricular preparation. As can be seen, addition of Gpp(NH)p transformed the isoproterenol displacement curve from one having a shallow slope to a curve having a steeper slope. The shift

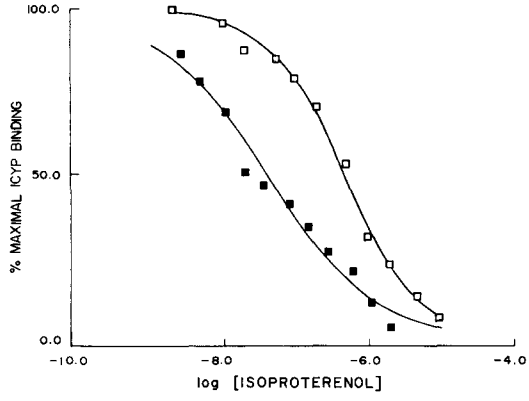

Fig. 1. Displacement of ICYP binding by increasing concentrations of isoproterenol in MR/Har left ventricular homogenate preparation in the absence (■) and presence (□) of 100 μM Gpp(NH)p.

seen in Fig. 1 was accompanied by a significant increase in the average IC_{50} and Hill slope values for both strains.

Features of agonist binding which distinguish the strains

Significant differences were observed between the two strains in the percentage of receptors found in the high-affinity state ($\%R_H$). Fifty-nine percent of the total receptor population was calculated to be in the high-affinity state in MR/Har ventricular preparations, with the remaining 41% in the low-affinity state. These findings were reversed in the MNRA/Har strain. Approximately, 59% of the cardiac ventricular β-receptors were in the low-affinity state and 41% in the high-affinity state (Table 1).

Guanyl nucleotide regulation

Examination of guanyl nucleotide concentration-response curves revealed that increasing concentrations of Gpp(NH)p decreased the ability of isoproterenol to displace ICYP from cardiac β-receptor binding sites of both strains (Fig. 2). The diminished ability of isoproterenol to displace ICYP in the presence of increasing Gpp(NH)p concentrations is related to the well documented effect of Gpp(NH)p to convert high-affinity binding sites into low-affinity binding sites in a concentration-dependent manner. Concentration–effect analysis of these curves demonstrated that the MR/HAR had a significantly lower EC_{50} value (for guanyl nucleotide inhibition of isoproterenol displacement of ICYP binding) of 1.2 μM, when compared to that of the MNRA/Har which was 2.5 μM ($P < 0.02$, 9 d.f., Student's t-test). However, there was no difference in the maximal effect of Gpp(NH)p on β-adrenoceptors in ventricular homogenates of the two strains, as 100% of

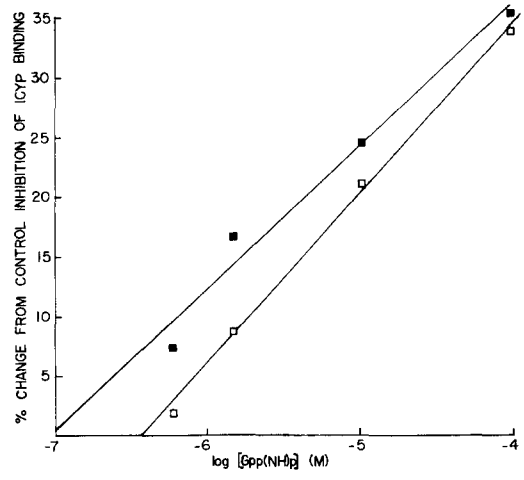

Fig. 2. Effect of increasing concentrations of Gpp(NH)p on isoproterenol (500 nM) competition for ICYP binding sites in MR/Har (■) and MNRA/Har (□) left ventricular homogenates. The control condition is defined as the displacement of 25 pM ICYP binding produced by a concentration of 500 nM isoproterenol in the absence of guanine nucleotide. Each point represents the mean of five determinations.

Table 1. Binding parameter estimates of agonist interaction with left ventricular β-receptor*

	Hill slope		IC_{50} (nM)			K_L (nM)				
	−Gpp(NH)p	+Gpp(NH)p	−Gpp(NH)p	+Gpp(NH)p	K_H (nM)	−Gpp(NH)p	+Gpp(NH)p	K_L/K_H	$\%R_H$
MR/Har	0.433 ± 0.022	0.829 ± 0.060*	37 ± 10	545 ± 96*	2.1 ± 0.46	256 ± 49.3	260 ± 34.7	143 ± 25.7	59 ± 3.3†
MNRA/Har	0.466 ± 0.032	0.823 ± 0.043*	47 ± 8.0	434 ± 54*	1.3 ± 0.37	157 ± 45.1	214 ± 12.9	140 ± 21.5	42 ± 4.5

Binding parameter estimates for isoproterenol displacement of ICYP binding in left ventricular homogenates were determined in the absence and presence of 100 μM Gpp(NH)p. In all cases, the Hill slope and IC_{50} values were shifted in the anticipated direction. The MR/Har was found to have a greater proportion of receptors in the high-affinity state ($\%R_H$) than the MNRA/Har. Each value is the mean ± SEM for $N = 9$.

* Significant difference between Gpp(NH)p present and absent conditions, $P < 0.0002$.

† Significant difference between inbred strains, $P < 0.006$.

the β-receptors in homogenates from both strains were shifted to a uniform, low-affinity state at the maximal Gpp(NH)p concentration (Fig. 2, Table 1).

Tissue catecholamine levels

Extending previous observations in whole heart [2], we found that the MR/Har also had lower levels of tissue catecholamines in left ventricle than the MNRA/Har. Left ventricular NE levels were found to be 12.19 ± 0.94 and 8.73 ± 0.95 ng/mg protein in the MNRA/Har and MR/Har strains respectively. The difference reported here is of the same magnitude as that reported earlier (MR/Har was ≈ 40% of MNRA/Har) [2]. When the previous results are corrected for protein instead of tissue wet weight, the values are remarkably similar to our current findings. In addition, we found that the MNRA/Har also had significantly ($P < 0.01$) higher levels of EPI in the left ventricle [0.165 ± 0.038 ng/mg protein (mean ± SEM, N = 8) for EPI in MNRA/Har and 0.018 ± 0.018 in MR/Har]. In the MR/Har, EPI could be detected in only one out of eight left ventricles assayed for catecholamines, while six out of eight left ventricles had detectable EPI levels in the MNRA/Har.

Addition of exogenous GTP and five homogenizations and resuspensions resulted in an ≈ 92% reduction in left ventricular NE levels, eliminating strain differences in tissue NE levels. There was no detectable EPI in homogenates from either strain following the homogenization procedure.

Features of agonist binding common to both strains

In the absence of Gpp(NH)p, isoproterenol competition for ICYP binding was indicative of two populations of receptors, of high and low affinity respectively, in both strains (Table 1). The dissociation constants for isoproterenol binding to the high (K_H) and low affinity (K_L) states were not different between the two strains. The K_L/K_H ratios (a proven index of intrinsic activity) in the absence of Gpp(NH)p in MR/Har and MNRA/Har left ventricular preparations were also found to be similar, and in agreement with K_L/K_H ratios for full agonists as determined in frog erythrocytes (Table 1) [5]. In the presence of a saturating concentration of Gpp(NH)p, isoproterenol displacement of ICYP binding modeled a homogeneous population of low-affinity receptors with similar K_L values in the two strains. Addition of Gpp(NH)p did not alter total ICYP binding.

DISCUSSION

Previous studies have demonstrated that MR/Har rats have a significantly greater β-receptor density in whole heart than MNRA/Har rats [4]. The present studies extend our earlier findings, in that MR/Har rats were found to have a significantly ($P < 0.05$) greater number of β-adrenoceptor binding sites than the MNRA/Har rats in left ventricle, thus giving a more precise indication of anatomic specificity. In contrast to our earlier work, these observations were made after removal of endogenous catecholamines by homogenization in the presence of exogenous GTP. Thus, strain differences in left ventricular β-receptor density are independent of residual tissue catecholamines and any effect that catecholamines might have on determination of B_{max} [9].

We also report here genetic differences in agonist binding with the β-adrenergic receptor in the absence of guanine nucleotides. The percent of receptors in the high-affinity state was found to be significantly ($P < 0.006$) higher in MR/Har male rats than MNRA/Har male rats. Our findings are consistent with the results of another recent study which reports an inability to form the high-affinity state of the receptor in senescence [14]. In this study, young rat hearts were found to have a calculated $\%R_H$ of 60 (similar to MR/Har), whereas hearts of older animals were found to have a $\%R_H$ of 40 (equivalent to MNRA/Har). It was postulated that this decrease in propensity to form the high-affinity state may account for the well documented decrease in myocardial responsivity to catecholamines seen with senescence [14]. Other investigators [15] have suggested that reduced coupling efficiency of the β-adrenoceptor in aging human tissue may account for the reduced sensitivity to β-agonists seen with advancing age. In addition, a decrease in the population of receptors capable of high-affinity state formation has been reported as a possible mechanism for the decreased myocardial function seen with experimentally induced diabetes in laboratory animals [16]. Thus, information from several investigations suggests that impaired high-affinity state formation may indeed translate into decreased *in vivo* β-adrenoceptor responsiveness.

The MNRA/Har was found to have significantly ($P < 0.01$) higher initial levels of both NE and EPI than the MR/Har in left ventricular homogenates. However, these strain differences in left ventricular catecholamine levels were abolished after addition of exogenous GTP and five successive resuspensions. Because the left ventricular catecholamine content is not different between the strains during assay conditions, we can conclude that the $\%R_H$ difference reported here is not an artifact of the effects of differential catecholamine levels in the two strains.

Analysis revealed that the EC_{50} for guanyl nucleotide inhibition of isoproterenol competition of ICYP binding sites (i.e. median dose) was significantly lower for the MR/Har when compared to the MNRA/Har (Fig. 2). Thus, for any given submaximal concentration of Gpp(NH)p, the effect of Gpp(NH)p on the β-adrenoceptor population in left ventricular homogenates would be greater on the MR/Har than the MNRA/Har, suggesting that G protein may be more efficiently coupled to the cardiac β-receptor of the MR/Har than the MNRA/Har. Under the binding assay conditions described here, we found an equivalent amount of radioactivity present in left ventricular homogenate preparations of the two strains, implying the presence of similar amounts of GTP and its metabolites. If the $\%R_H$ differences reported above were a function of differential GTP sensitivity, then one would have expected the GTP sensitivities detected to be the opposite of those described here.

The potential complications introduced into interpretation of agonist binding to the β-adrenoceptor by the presence of catecholamines or residual GTP

in the preparation must be carefully considered in future studies, and may well have played a role in previous experimental findings. Comparison of tissue homogenization in the absence and presence of GTP confirmed the findings of Nerme *et al.* [9], in that we found a significantly higher B_{max} when tissues were homogenized with GTP. Homogenization in the presence of high quantities of GTP necessitates its removal before conducting agonist competition studies. Our results show that at least five rinses are necessary before GTP levels are sufficiently reduced. Thus, our tissue preparation made it possible to exclude differential levels of endogenous catecholamines, as well as differential GTP sensitivity and/or concentrations (as approximated from the disappearance of radioactivity) as the cause of the $\%R_H$ differences between the strains. Instead, $\%R_H$ may well be a reflection of intrinsic differences in the receptor complex.

If one multiplies the calculated $\%R_H$ for each strain by the detected B_{max} for that strain, the MR/Har has almost two times the number of receptors capable of high-affinity state formation as the MNRA/Har (i.e. theoretically, a maximum of 7.79 fmol receptor/mg protein for the MR/Har, and 4.49 fmol receptor/mg protein for the MNRA/Har could form the high-affinity state). Perhaps this discrepancy in high-affinity state formation, which has been demonstrated to be related to biological response, and postulated as the mechanism responsible for altered β-adrenergic responsiveness in other studies (as discussed above), could account for some of the *in vivo* differences reported in resting heart rate and blood pressure between the two strains. Further studies will be necessary to demonstrate the *in vivo* biological significance of the differences reported here.

The importance of using computer modeling techniques for analyzing radioligand receptor binding is highlighted in these studies. The results of an analysis of the Hill coefficients and IC_{50} values alone would have provided no new insights concerning possible differences in β-receptor function in two rat strains with well documented differences in autonomic function.

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