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DSP4-Induced Noradrenergic Lesions Increase β-Adrenergic Receptors and Hippocampal Electrophysiological Responsiveness[1]

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ZAHNISER, N. R., G. R. WEINER, T. WORTH, K. PHILPOTT, R. P. YASUDA, G. JONSSON AND T. V. DUNWIDDIE. *DSP4-induced noradrenergic lesions increase β-adrenergic receptors and hippocampal electrophysiological responsiveness.* PHARMACOL BIOCHEM BEHAV **24**(5) 1397–1402, 1986.—Following profound (>90%) depletions of norepinephrine (NE) by the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4), the numbers of β-adrenergic receptors were significantly increased (20–25%) in rat hippocampal and somatosensory cortical membranes; however, the numbers of α_1-adrenergic receptors and the affinities of both types of receptors were unaffected. This selective up-regulation of β-adrenergic receptors was evident 1 week after DSP4 administration and was maintained for at least 2 more weeks. In electrophysiological experiments in the hippocampal slice preparation, responses to threshold as well as maximal concentrations of isoproterenol were enhanced 150% and 33%, respectively, in the DSP4-lesioned animals. The results demonstrate that nearly complete depletion of brain NE produced by administration of DSP4, like that produced by 6-hydroxydopamine, results in increased numbers of β- but not α-adrenergic receptors, and suggest that the density of the former are regulated by afferent noradrenergic fibers. Furthermore, the functional significance of the increased number of hippocampal β-adrenergic receptors is directly manifested in a greater electrophysiological responsiveness to an exogenously administered β-adrenergic receptor agonist.

Adrenergic receptors (α- and β-), regulation β-Adrenergic receptors, electrophysiological responsiveness
DSP4 lesions Hippocampal slices

DENERVATION or chronic receptor blockade frequently elicit compensatory changes in noradrenergic neurons and their targets. These may include changes in transmitter synthesis, release, receptor number and cAMP formation (see [4, 14, 26]). The functional consequences of an up-regulation of postsynaptic β-adrenergic receptors have been examined in the brain using a number of paradigms. For example, one week after 6-hydroxydopamine (6-OHDA) induced lesions, the production of cAMP in response to isoproterenol is elevated by 80% as a result of a 30% increase in the number of cerebrocortical β-adrenergic receptors [25]. However, the electrophysiological sequelae of noradrenergic lesions are less well characterized; responses to iontophoretically applied norepinephrine (NE) in the cerebellum and hippocampus show increases in magnitude and/or duration following 6-OHDA administration [7, 12, 17, 22], but this increased sensitivity might well result from diminished uptake of NE consequent to the loss of noradrenergic nerve terminals, and thus would not reflect postsynaptic supersensitiv-

ABBREVIATIONS

2-(β-(4-hydroxyphenyl)-ethylaminomethyl)-tetralone (BE 2254)
6-hydroxydopamine (6-OHDA)
[^{125}I]-(−)cyanopindolol (ICYP)
N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4)
norepinephrine (NE)

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ity. Electrophysiological increases in adrenergic sensitivity directly linked to an up-regulation of central β-adrenergic receptors following noradrenergic lesions have not been reported.

In order to examine the functional consequences of a noradrenergic lesion, it is advantageous to use a neurotoxin specific for noradrenergic neurons. Jonsson and colleagues [13] demonstrated that administration of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) produces a selective loss of NE without affecting levels of dopamine. However, in a previous study we found no effect of DSP4 treatment on either β-adrenergic receptors or electrophysiological sensitivity to β-adrenergic agonists, despite an average 73% depletion in levels of hippocampal NE [8]. A possible explanation for this lack of effect is that there exists a threshold for depletion below which changes in receptors do not occur; in the nigrostriatal dopamine system, this threshold has been reported to be >90% loss of striatal tyrosine hydroxylase activity for receptor changes [5] and >80–90% losses of striatal dopamine content for functional postsynaptic changes [11,30].

In the present communication we have characterized several important aspects of the relationship between presynaptic noradrenergic nerve terminals, postsynaptic receptors, and the responses that are mediated by these receptors. First, we have shown that more complete depletions of NE (>90%) produced by DSP4 do induce a consistent up-regulation of β-adrenergic receptors in two brain areas that are innervated by dorsal bundle fibers, cerebral cortex and hippocampus. Additionally, we have determined which populations of adrenergic receptors are regulated by the presence of presynaptic nerve terminals. Finally, using the *in vitro* hippocampal slice preparation, we have characterized the functional electrophysiological consequences of noradrenergic lesions. The results suggest that the up-regulation of β-adrenergic receptors is functionally relevant because it is translated into an enhanced electrophysiological responsiveness to a β-adrenergic receptor agonist in the rat hippocampus.

METHOD

Animals and Drug Treatment

Male Sprague-Dawley rats (150–250 g) were obtained from Sasco (Omaha, NE) and were maintained on a 12-hr light-dark cycle with chow and water ad lib. DSP4-HCl was dissolved in saline and injected into the rats at a concentration of 50 mg/kg (IP). Care was taken to inject rats within 5 min of making up the DSP4 solution; this is important so that a minimal amount of cyclization of DSP4 to the charged aziridinium ion occurs prior to injection [20] and DSP4 is able to freely penetrate the blood-brain barrier. Controls received an equal volume (0.5 ml) of saline. Rats were killed by decapitation 1, 2 and 3 weeks after DSP4 administration.

Catecholamine Levels

Tissue levels of NE and dopamine were determined by liquid chromatography and electrochemical detection (LCEC Application Notes No. 12 and 14, Bioanalytical Systems, West Lafayette, IN). Tissues (10–15 mg) were weighed; added to 400 μl 0.1 M perchloric acid (pH 1) containing 1.3 mM Na_2EDTA, 7.9 mM Na-metabisulfite and dihydroxybenzylamine (0.14 μM for NE and 0.29 μM for dopamine samples; internal standard); frozen in a dry-ice acetone slurry and stored at −70°C. Samples were thawed, homogenized by sonication and centrifuged at 30,000 × g for 10 min at 4°C. Catecholamines were isolated from the supernatant onto acid-washed alumina by gentle shaking for 10 min in 1.5 vol of 1.5 M Tris-HCl buffer (pH 8.6) containing 1.3 mM Na_2EDTA and 7.9 mM Na-metabisulfite. The alumina particles were precipitated and the supernatant was discarded. After washing the alumina twice more, the catecholamines were eluted from the particles by gentle shaking in 480 μl of 0.1 M perchloric acid. The mobile phase, 0.15 M monochloroacetic acid (pH 3) containing 2 mM Na_2EDTA and 0.56 mM Na-octylsulfate, had a flow rate of 1.5 ml/min. The temperature of the Biophase ODS column (5 μm) was 20°C, and the electrode potential was 0.65 V. The recovery of NE and dopamine was approximately 40%.

Receptor Binding Studies

For the β-adrenergic receptor studies, somatosensory cortex and hippocampus were homogenized with a Polytron (10 sec; speed 5) and membranes isolated by centrifugation at 20,000 × g and 4°C for 10 min in 0.32 M sucrose buffered with 10 mM Tris-HCl (pH 7.5) and washed by a similar centrifugation in 154 mM NaCl buffered with 20 mM HEPES (pH 7.5). Final resuspension of the washed membranes was at a concentration of 50–100 μg protein/ml in NaCl/HEPES buffer. (−)Cyanopindolol was radiolabeled with Na[^{125}I] according to the procedure of Engel *et al.* [10] to form [^{125}I]-(−)cyanopindolol (ICYP; specific activity=2.2 Ci/μmol). Assays contained 50 μl ICYP (2.5–180 pM final concentration), 50 μl 1 mM HCl, 50 μl HCl or l-propranolol and 100 μl tissue homogenate. Specific binding was defined as the difference in ICYP bound in the absence and presence of 1 μM l-propranolol. Assays were incubated for 2 hr at 37°C. Reactions were terminated by dilution with 10 ml 154 mM NaCl buffered with 10 mM Tris-HCl (pH 7.5) at 37°C and rapid filtration over glass fiber filters (Schleicher and Schuell No. 30). Filters were washed with an additional 10 ml of NaCl/Tris buffer.

For α_1-adrenergic receptor assays, membranes were isolated from the tissues in the same manner as above with the exception that the wash and final resuspension were in 20 mM Na-phosphate buffer (pH 7.4) containing 154 mM NaCl [16]. The final resuspension was at a concentration of 100–200 μg protein/ml. 2-(β-(4-Hydroxyphenyl)-ethylaminomethyl)-tetralone (BE 2254) was radioiodinated to [^{125}I]-BE 2254 (IBE 2254; specific activity=2.2 Ci/μmol) as described by Engel and Hoyer [9]. Assays contained 50 μl IBE 2254 (10–300 pM final concentration), 50 μl 1 mM HCl, 50 μl HCl or prazosin and 100 μl membrane homogenate. Specific binding was defined as the difference in the IBE 2254 bound in the absence and presence of 1 μM prazosin. Assays were incubated for 90 min at 20°C. Reactions were terminated and bound radioligand was separated from free as detailed above with the exception that ice-cold buffer was used.

Saturation curves were analyzed by the method of Scatchard [21]. The transformed data resulted in linear plots and were used to determine the density of binding sites (Bmax) and the equilibrium dissociation constants (Kd values). Statistical significance between mean values was assessed using a two-tailed Student's *t*-test. Protein concentrations were determined by the dye-binding method of Bradford [2] using bovine serum albumin as the standard. Sodium hydroxide (0.1 N) was added to each sample to solubilize the proteins prior to assay.

TABLE 1

EXTENT OF TISSUE CATECHOLAMINE DEPLETIONS AND PARAMETERS OF β- AND α_1-ADRENERGIC RECEPTOR BINDING IN RAT CEREBROCORTICAL AND HIPPOCAMPAL MEMBRANES 1 AND 2 WEEKS FOLLOWING DSP4 ADMINISTRATION

Brain Area	Treatment Time (weeks)	Treatment Group	[CA] (ng/g)	ICYP Bmax (fmol/mg prot)	ICYP Kd Value (pM)	IBE 2254 Bmax (fmol/mg prot)	IBE 2254 Kd Value (pM)
Cortex	1	Control	310 ± 16	61 ± 1.4	2.8 ± 0.20	140 ± 9.6	32 ± 4.5
		DSP4	25 ± 5.3‡	74 ± 1.5‡	3.0 ± 0.24	140 ± 10	29 ± 5.6
	2	Control	300 ± 34	57 ± 1.6	3.4 ± 0.11	160 ± 10	18 ± 1.4
		DSP4	27 ± 11‡	72 ± 2.6‡	3.5 ± 0.10	190 ± 14	21 ± 1.1
Hippocampus	1	Control	450 ± 40	39 ± 1.5	5.8 ± 1.1	59 ± 6.8	87 ± 22
		DSP4	29 ± 5.1‡	48 ± 1.7†	5.8 ± 0.81	56 ± 5.9	68 ± 12
	2	Control	590 ± 44	42 ± 1.8	8.4 ± 0.37	80 ± 5.6	21 ± 2.4
		DSP4	49 ± 11‡	50 ± 2.3*	8.1 ± 0.63	77 ± 2.8	18 ± 1.4
Striatum	1	Control	9800 ± 930	—	—	—	—
		DSP4	10000 ± 480	—	—	—	—

Mean values ± SEM for N=5–7 animals.
CA=tissue level of NE for cortex and hippocampus and of dopamine for striatum.
*$p<0.05$ compared with control.
†$p<0.01$.
‡$p<0.001$.

Electrophysiological Studies

Brain slices for *in vitro* electrophysiological recording were prepared from rat hippocampus as described previously [8,18]. Coronal slices of hippocampus (400 μm) were allowed to recover for 1 hr at 34°C in oxygenated (95% O_2/5% CO_2) Krebs' buffer consisting of 124 mM NaCl, 4.9 mM KCl, 1.2 mM KH_2PO_4, 2.4 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25.6 mM $NaHCO_3$ and 10 mM glucose (pH 7.6). Slices from control and lesioned animals were run simultaneously in the same chamber and were subsequently analyzed as paired measurements. Synaptic responses were evoked by stimulating the Schaffer collateral and commissural afferents to the CA1 region of the hippocampus at 1 min intervals and recording the evoked population spike responses from the cell body layer. As in previous studies, the stimulus intensity was adjusted so that a population spike of approximately 25% of the maximal response was elicited; under these conditions, β-adrenergic receptor agonists elicit marked increases in population spike amplitude. Slices were then superfused with l-isoproterenol (10–500 nM), and the maximal increase in the population spike response in the succeeding 10 min was determined. Isoproterenol concentrations less than 50 nM did not elicit significant changes in population spike amplitude (N=16 slices), and the maximal response in both lesioned and control slices was observed at 250 nM; thus, most of the slices were tested at the threshold (50 nM) and maximal (250 nM) drug concentrations. Responses elicited from different slices were combined by expressing the population spike amplitude during the isoproterenol perfusion as a percentage of predrug response. Statistical significance between mean values was assessed using a paired *t*-test, and differences in the dose response curves were determined with a 2-way analysis of variance.

Drugs

(−)Cyanopindolol and BE 2254 were kindly given to us by Dr. G. Engel, Sandoz Ltd., Basel, Switzerland. Other drugs were donated by the following sources: l-propranolol by Ayerst Laboratories Inc., New York, NY; prazosin by Pfizer Laboratories, New York, NY; and l-NE by Sterling-Winthrop Research Institute, Rensselaer, NY. All other drugs and chemicals were of the highest purity commercially obtainable.

RESULTS

One to three weeks after a single intraperitoneal injection of DSP4 (50 mg/kg), forebrain NE content was markedly reduced. By 1 week after administration of DSP4, NE levels were decreased by 92% in the cerebral cortex and by 94% in the hippocampus (Table 1). In contrast, in these same two groups of animals the levels of dopamine in the striatum remained unchanged (Table 1).

Following DSP4 administration, there were small (19–26%) but significant increases in the numbers of β-adrenergic receptors as measured with ICYP binding to membranes prepared from both somatosensory cortex and hippocampus (Table 1). Scatchard plots were linear and demonstrated that the changes in ICYP binding were restricted to the numbers of β-adrenergic receptors; no differences in the affinities of the receptors were observed between control and lesioned groups (Fig. 1 and Table 1). The numbers of β-adrenergic receptors in rat cortical and hippocampal membranes were increased by 1 week after DSP4 administration and remained elevated until at least 3 weeks post-lesion (Fig. 2). An up-regulation of cortical and hippocampal β-receptors was observed in all five experiments conducted; the depletions of tissue NE ranged from 91–94% in these experiments.

Despite the profound depletion of NE and the up-regulation of β-adrenergic receptors in somatosensory cortex and hippocampus, however, no significant changes in the properties of α_1-adrenergic receptors were detected in these two brain areas 1–2 weeks post DSP4 administration (Table 1). Scatchard plots of IBE 2254 binding were also consistent with binding to a single homogenous population of binding

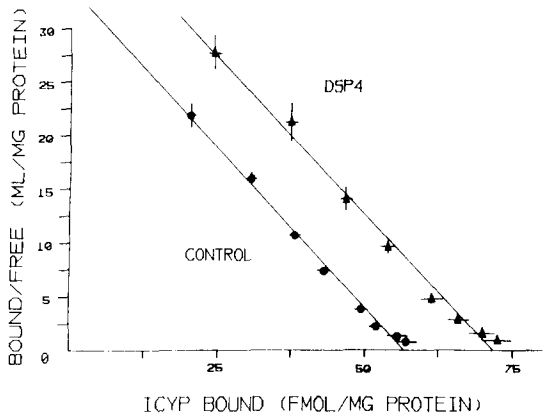

FIG. 1. Scatchard plot of the binding of ICYP to β-adrenergic receptors on cortical membranes from rats treated 2 weeks prior with DSP4. Saturation plots were constructed using a range of concentrations of ICYP from 2.8 to 160 pM. The values shown are mean values±SEM for N=7 for the control and N=5 for the DSP4-treated rats.

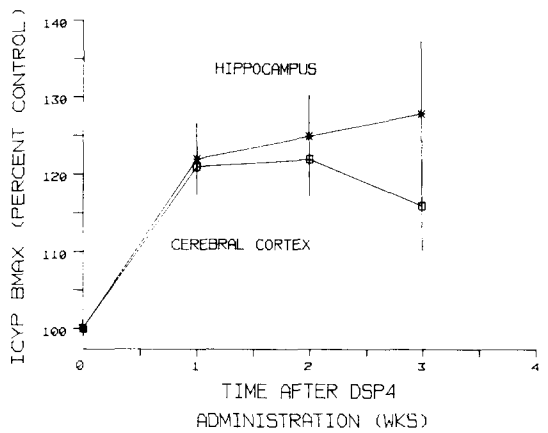

FIG. 2. Time course of the elevation in numbers of β-adrenergic receptors in rat cortical (□) and hippocampal (*) membranes in response to DSP4 administration. Tissue levels of NE were depleted by >90%. The Bmax values for ICYP binding were calculated from Scatchard analysis and are expressed as a percent of untreated control values±SEM for N=4–15 animals at each point. The mean numbers of β-adrenergic receptors 1, 2 and 3 weeks after DSP4 treatment were respectively 61±1.4 fmol/mg protein, 49±2.2 and 68±2.0 in cortical membranes and 39±1.3, 31±2.6 and 34±3.9 in hippocampal membranes.

sites. Therefore, regulation of adrenergic receptors, induced by large depletions of NE in rat cortex and hippocampus in the first two weeks following DSP4 administration, was restricted to β-adrenergic receptors.

In the *in vitro* hippocampal slice preparation, superfusion with β-adrenergic receptor agonists has been shown to increase the amplitude of synaptically-evoked population spikes [18]. This paradigm was used to determine whether an increased number of β-adrenergic receptors in the hippocampus altered functional responses to a β-adrenergic agonist. In these experiments we tested the effects of l-isoproterenol because it is not a good substrate for the high affinity neuronal uptake pump. We have previously shown that the apparent potency of isoproterenol is not affected by the loss of this uptake in hippocampal slices [29]. Dose-response curves to l-isoproterenol were generated in hippocampal slices from control animals and in a group of lesioned animals in which there was >90% depletion of NE in the hippocampus (control NE content=340±53 ng/g tissue, DSP4-treated=33±13 ng/g). Isoproterenol concentrations ranging from 10–500 nM were tested. An analysis of variance on the results demonstrated a significantly greater sensitivity in the DSP4-treated group ($F=9.33$, $p<0.003$; N=108 slices from 12 animals). To further characterize the differences in responsiveness, slices from a second group of animals were tested at the threshold concentration of isoproterenol (50 nM), and the concentration that elicited a maximal response (Fig. 3). Defining a maximal response to isoproterenol is somewhat difficult, because higher concentrations elicit highly variable, and in some cases depressant responses [1,18]. For this reason, slices were tested with the concentration (250 nM) that had produced the maximal response in slices from both control and DSP4-treated animals in our initial dose-response studies. Both concentrations elicited significantly greater responses in the DSP4 lesioned animals than in controls with the maximal responsiveness being increased by 33% following DSP4 treatment (Fig. 3).

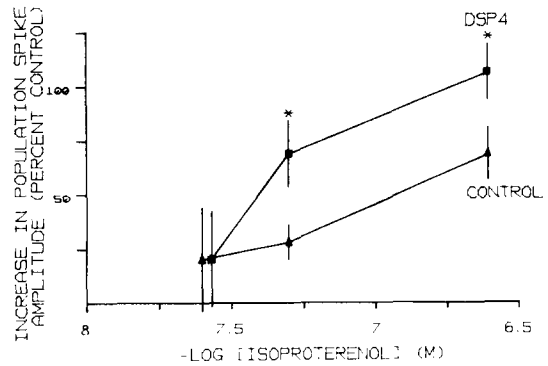

FIG. 3. Sensitivity to isoproterenol as measured electrophysiologically in the rat hippocampal slice preparation 2–3 weeks after DSP4 administration. Depletions of NE were >95%. Points represent mean values for 6–21 slices from 8 animals. Higher concentrations of isoproterenol (≥500 nM; not shown) elicited smaller responses than did 250 nM in both groups. Statistical significance (*) was assessed using a 2-tailed Student's *t*-test with paired observations.

DISCUSSION

The results presented here indicate that extensive depletions of NE lead to an increased number of β-adrenergic receptors in both the cerebral cortex and the hippocampus. Although significant changes were observed in β-adrenergic receptors, neither somatosensory cortex nor hippocampus showed significant changes in α_1-adrenergic receptors. Additionally, in a single experiment, the properties of α_2-adrenergic receptors were measured using [^{3}H]-rauwolscine binding to cortical and hippocampal membranes 1 week after DSP4 administration. Again, no alterations in the properties of these receptors in either of these tissues were observed (data not shown). These results are consistent with previous studies in which cortical (not including frontal cortex) and hippocampal α_1 receptors were unaltered following extensive depletions of NE induced by 6-OHDA [23, 24, 28]. On

the other hand, other investigators have reported increases in central α_1 and/or α_2 receptors following 6-OHDA administration [6, 15, 19, 27]. The explanation for such inconsistent effects upon α_1 receptors is not known; however, Sutin and Minneman [27] have recently demonstrated that, of all the cortical areas examined, somatosensory cortex, which we examined in the present studies, is the only one in which α_1 receptors are not up-regulated as a consequence of neonatal 6-OHDA treatment. Nevertheless, lack of an effect on these receptors does not preclude a functional change that may ensue without an altered receptor number. The α_1 receptor agonists methoxamine and l-phenylephrine do not induce electrophysiological responses in hippocampal brain slices [18], so we were unable to test this possibility. Menkes and colleagues [15], however, have correlated the up-regulation of thalamic α_1 receptors with a selective increase in sensitivity of lateral geniculate neurons to α_1 receptor agonists. The present results suggest that the numbers of β- but not α-adrenergic receptors are regulated by the presence of noradrenergic nerve terminals in hippocampus.

In terms of changes in β-adrenergic receptors, both DSP4 and 6-OHDA have been previously reported to produce increases in receptor number [3, 13, 23, 24, 25, 28]. The electrophysiological measurements of hippocampal β-adrenergic sensitivity appear to be somewhat similar to other functional measures of receptor activation in that the magnitude of the increase in the number of receptors (approximately 20%) was somewhat less than the increase in the population spike response to a maximal concentration of isoproterenol (approximately 33%) and considerably less than the increase in response to a threshold concentration of isoproterenol (approximately 150%). For example, disparities between the increase in receptor numbers and accumulation of cAMP have been reported [24,25], and have lead to the suggestion that changes in receptor number are amplified during the chain of events producing functional responses. Regarding the changes in receptors, in a previous study we demonstrated that smaller depletions of hippocampal NE (average 73%) induced no significant change in β-adrenergic receptors or electrophysiological responses to isoproterenol [8]. In combination with the present findings, it would appear that there may be a critical threshold level of NE depletion that is required to induce significant changes in receptor number (or in sensitivity to isoproterenol), and that this threshold is between a 75–90% loss of NE. This finding is similar to what is observed in the nigrostriatal system, where dopamine receptor and functional postsynaptic changes occur only when >80–90% of the striatal tyrosine hydroxylase activity or dopamine content is depleted [5, 11, 30].

Additionally, we have clearly demonstrated in the hippocampus that the up-regulation of β-adrenergic receptors is correlated with an enhanced electrophysiological responsiveness to the β-adrenergic receptor agonist isoproterenol. Previous studies have shown increased sensitivity to the effects of NE following 6-OHDA lesions [22]; however, isoproterenol was not tested. We have recently demonstrated that large changes in apparent sensitivity to NE can occur as a result of changes in uptake with no change in postsynaptic receptors [29]. Thus, electrophysiological responses to exogenous NE can be affected by loss of the reuptake pump as well as by changes in the number of postsynaptic β-adrenergic receptors following lesions of the presynaptic nerve terminals. By utilizing a β-adrenergic receptor agonist that is not a substrate for uptake, it is likely that the increased sensitivity observed in the present experiments reflects postsynaptic receptor changes and not uptake. These findings complement a recent study in which decreases in the sensitivity of hippocampal brain slices to isoproterenol were reported following chronic treatment with antidepressants [1], a treatment that has been reported to reduce β-adrenergic receptor number. Thus, the hippocampal slice provides a sensitive system in which to test altered responsiveness to β receptor activation following a variety of pharmacological treatments. Together with our previous results in this system, these experiments suggest that receptor and related functional alterations do not normally occur unless the function of this system is severely compromised; when smaller functional deficits develop, whatever compensation that occurs appears to be largely pre- rather than postsynaptic in nature.

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