none of these changes was statistically significant. The activity of the CAF protease (change in absorbance per g wet muscle weight) was not different among treatment groups (table). Similar nonsignificant increases were observed when CAF was expressed as the change in absorbance per mg of protein isolated or as the absolute change in absorbance (data not shown).

Discussion. The increased excretion of urinary 3MHIS observed after submaximal exercise is consistent with the hypothesis that muscle protein degradation was elevated. These results are in agreement with previous evidence of increased rates of muscle protein degradation after maximal exercise[1]. However, the only significant structural alteration that occurred in m. triceps brachii after this submaximal bout of eccentric exercise was the increased relative percentage of the 125,000 d protein 18 h after the exercise. The protein accounted for only 1.5% of the minor myofibrillar proteins. This minor change observed in the white triceps brachii is consistent with the data of Armstrong et al.[9] who showed an increased activity of glucose-6-phosphate dehydrogenase, a marker of muscle inflammation, in red muscles after an exercise bout similar to the one used in this study, but not in the triceps brachii. Similarly, Kuipers et al.[14] found degenerative changes to be greatest in red muscle fibers of rats 24–48 h after a submaximal nonexhaustive 1-h exercise, and Vihko and coworkers[15] found proteolytic lysosomal enzyme activity of rats to be increased more in red muscle than white following strenuous exercise. Finally, Dohm et al.[1] found no increase in the activity of CAF in rat gastrocnemius muscle, a white muscle, immediately following a run to exhaustion. Since the activity of CAF[16] and other proteolytic enzymes[17] has been found to be greater in red than white muscle, it appears likely that increased muscle protein degradation and structural alterations following exercise occur initially and preferentially in the red muscle fibers.

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Evaluation of monoaminergic receptors in the genetically epilepsy prone rat

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Summary. The intensity of sound-induced convulsions in the genetically epilepsy-prone rat (GEPR) was reduced in a dose related fashion by intracerebroventricular administration of dobutamine, (β_1 agonist), terbutaline (β_2 agonist) or phenylephrine (α_1 agonist). BHT-920 (α_2 agonist) did not cause a dose-related decrease in sound-induced convulsion intensity. Binding studies showed that whole brain α and β receptor densities (B_{max}) were normal while the K_d was increased for the β ligand in GEPR brain.

The genetically epilepsy-prone rat (GEPR) has a lower seizure threshold and more intense seizures for a given stimulus (electroshock, pentylenetetrazol or bicuculline) than do normal rats[3–5]. These rats are also characterized by susceptibility to sound-induced seizures[6] and to hyperthermic seizures[7]. Thus, like the human epileptic, the GEPR has a genetically determined decreased ability to suppress seizures once the process has been initiated[8].

Perturbation of biogenic amine neurotransmitter systems has been shown to alter seizures in a number of animal models although innate abnormalities in these systems have not been conclusively identified in most models (see Maynet et al.[9] and Chadwick[10] for reviews). In the GEPR, experimentally-induced perturbation of biogenic amines alters seizure characteristics and innate abnormalities in monoamine neurotransmitters have been demonstrated. Drug-induced decrements in CNS noradrenergic and serotonergic activity cause an increase in sound-induced seizure intensity in the GEPR[11–17]. Drug-induced increments in noradrenergic and/or serotonergic activity are associated with a decrease in the severity of sound-induced seizures[12,14,18–20]. Also, when compared with controls, the GEPR has a generalized abnormality in brain norepinephrine levels and turnover rate and widespread abnormalities in serotonin levels in the brain[20,21].

The purpose of the present investigation was 2-fold. First, it was to evaluate brain noradrenergic receptors by determining if selected noradrenergic agonists can decrease seizure

intensity in the GEPR. Secondly, it was to estimate the affinities and densities of noradrenergic receptors in the GEPR brain.

Methods. Animals used in these studies were male (280–350 g) and female (200–240 g) rats obtained from the colonies of seizure susceptible GEPRs and controls housed at the Veterans Administration Medical Center in Shreveport. Each of these colonies was derived from Sprague-Dawley (Madison, Wisconsin) stock through a selective breeding process. The GEPR colony was originated by screening rats until a male and a female with susceptibility to sound-induced seizures were found. These rats were bred to each other. From their progeny a process of brother to sister inbreeding was used to develop the present colony which has more than 90% of the progeny exhibiting susceptibility to sound-induced seizures. The control colony was developed by breeding only animals known to be resistant to sound-induced seizures.

Before being used in the present study, all GEPRs had exhibited sound-induced seizures in each of 3 separate tests carried out at weekly intervals. Controls did not exhibit convulsions in any of the 3 separate tests. Convulsive intensity was determined through use of the Audiogenic Response Score (ARS) system developed by Jobe et al.[12] and depicted diagrammatically in figure 1. According to this intensity rating scale, the GEPRs used in these experiments had an ARS of 3 and the controls had an ARS of 0 in each of their 3 weekly tests.

Sound stimulation was carried out as previously described[12]. Rats were placed into a cyclindrical metal chamber (40 cm in diameter by 50 cm high), a sound stimulus (124 db relative to 2×10^{-4} dyne/cm^2) was generated by 2 electric bells mounted at the top of the chamber and the animal behavior was observed through a glass port. The sound stimulus was administered to individual rats within 15 sec of placing the animal into the chamber and the stimulus was turned off at the onset of convulsion or after a stimulus duration of 90 sec if no convulsion occurred.

GEPRs were prepared for intracerebroventricular administration (right lateral ventricle) of adrenergic agonists by a modification of the technique of Vester et al.[22]. Drugs were dissolved in 0.9% saline and injected ICV in a total volume of 15 µl over a 60-sec time period. 30 min after the injection, the animals were subjected to the standardized sound stimulus and the intensity of their convulsion was rated. Preliminary experiments showed that vehicle injections did not affect the characteristics of the convulsion. The adrenergic agonists used were: phenylephrine HCl (an α_1 agonist[23]), BHT-920 (an α_2 agonist[24]) dobutamine HCl (a β_1 agonist[25]) and terbutaline sulfate (a β_2 agonist[25]). Preliminary dose-ranging experiments allowed selection of drug doses that suppressed convulsions in some, but not all animals so that the linear portion of the dose-response curves was generated for ED_{50} determinations by the method of Litchfield and Wilcoxan[26]. Dose response curves were drawn with the aid of a least squares regression analyses[27]. Each rat was used only once in the experiments.

Receptor binding assays were carried out in whole brains (excluding the cerebellum) from controls and from GEPRs. α-Receptors were evaluated using H^3-WB-4101 as the labeled ligand and phentolamine hydrochloride as the cold displacer. β-Receptors were evaluated using H^3 dihydroalprenolol and l-alprenolol-d-tartrate as the cold displacor. Tissue preparations and binding assays were carried out as

ARS score		Characteristic convulsive posture
0	No response.	
1	Running only; no convulsion.	
2	Two running phases separated by a refractory period; convulsive endpoint consists of clonus of forelimbs, hindlimbs, head, pinnae, vibrissae, and tail. (A)	
3	Same as 2 except only one running phase and no refractory period.	
4	Two running phases separated by a refractory period; convulsive endpoint consists of tonic flexion of neck, trunk, and forelimbs with clonus of hindlimbs. (B)	
5	Same as 4 except one running phase and no refractory period.	
6	Two running phases separated by a refractory period; convulsive endpoint similar to 4 except hindlimbs are in paritial tonic extension (i.e., tonic extension of thighs and legs with clonus of feet.) (C)	
7	Same as 6 except only one running phase and no refractory period.	
8	Two running phases separated by a refractory period; convulsive endpoint similar to 4 except hindlimbs are in complete tonic extension (i.e., animal in maximal convulsion). (D)	
9	Same as 8 except only one running phase and no refractory period.	

Figure 1. Description of the audiogenic response scoring (ARS) system for evaluation of convulsion intensity.

described by Bylund and Snyder[28]. Proteins were determined according to the method of Lowery et al.[29]. Receptor binding parameters were calculated by Scatchard analysis[30].

Results. Phenylephrine (α_1 agonist), dobutamine (β_1 agonist) and terbutaline (β_2 agonist) each decreased sound-induced convulsions in a dose related fashion in the GEPR (fig. 2). That is, these agonists caused decreases in the ARS ratings that were significantly correlated with the agonist dose ($p < 0.05$). BHT-920 (α_2 agonist) did not produce a statistically significant correlation ($p < 0.2$) between drug dosage and ARS ratings. BHT-920 doses above 20 µmoles/rat caused death in all of the animals within 30 min so that they could not be tested for convulsion intensity at doses above 20 µmoles. Based on ED_{50} calculations, dobutamine (ED_{50} 0.93 µmoles) was slightly more potent than phenylephrine (ED_{50} 1.43 µmoles) which in turn was more potent than terbutaline (ED_{50} 2.80 µmoles).

The table shows the affinities (K_d) and densities (B_{max}) of catecholamine receptors in control and GEPR brain. The only statistically significant difference between the groups was in the K_d for dihydroalprenolol with the GEPR having a higher ($p < 0.02$) K_d for this β ligand than did control.

Discussion. It is well accepted that peripheral organs innervated by the sympathetic nervous system have different types of receptors. The physiologic and pharmacologic effects of epinephrine and norepinephrine are mediated through these α and β in the periphery and perhaps in the CNS[31-37]. Previous studies in our laboratories have shown that drugs or procedures that cause an increase in synaptic norepinephrine also decrease the intensity of sound-induced convulsions in the GEPR[11,12,20]. In the present study, noradrenergic agonists with selectivity for particular receptor types, were administered to GEPRs in order to make an initial judgement about which type of noradrenergic receptor might be involved in the seizure suppressing actions of norepinephrine. Figure 2 shows dose-response curves for the convulsion suppressing activity of noradrenergic agonist. For 3 of the drugs (dobutamine, phenylephrine and terbutaline) there was a statistically significant correlation between drug dose and convulsive intensity (ARS). In other words, each of these 3 drugs caused a decrease in convulsion intensity that was correlated with drug dosage. For the 4th drug, BHT-920, there was no statistically significant correlation between drug dosage and ARS. These data are consistent with 2 hypotheses. The first is that there are 3 types of CNS adrenergic receptors which mediate the seizure suppressing actions of norepinephrine in the GEPR. These are an α_1 receptor, a β_1 receptor and a β_2 receptor which are selectively activated by phenylephrine, dobutamine and terbutaline respectively. Alternatively, there may be a single CNS adrenergic receptor type which mediates the convulsion suppressing actions of norepinephrine, phenylephrine, dobutamine and terbutaline. If a single CNS adrenergic convulsion-suppressing receptor exists, its characteristics may differ from those of the classic α and β receptor types that exist in association with the peripheral sympathetic nervous system. This is suggested by the fact that the 3 active agonists have ED_{50} doses for convulsion suppression that differ approximately 3-fold whereas if they each acted through classic α or β receptors, one would expect the range of ED_{50} doses to be considerably greater[25]. Further studies are needed to determine which of these 2 hypotheses is correct.

In a 2nd type of experiment, catecholamine receptors were evaluated in controls and in the GEPR by determining the affinities (K_d) and densities (β_{max}) of binding sites for α adrenergic, and β adrenergic ligands. The H_3-WB-4101 binding, which reportedly represents post synaptic α-adrenergic receptor binding in brain[37], was not different in control and GEPR brains. β_{max} for H^3 dihydroalprenolol, a nonspecific β agonist[38] was also similar for GEPR and control brains. However, there was a statistically significant difference between control and GEPR K_d-values for H^3 dihydroalprenolol binding (table). The K_d-value for GEPR brain binding was higher than for control. Thus, the affinity of this agonist for receptors in the GEPR brain appears to be lower than in control. This finding suggests that an abnormality may exist in the receptor that binds H^3 dihydroalprenolol in GEPR brain. At this time, it is not possible to say whether this altered affinity is, in any way, responsible for the seizure prone state in the GEPR. Conceivably, this alteration could have resulted from the seizure itself or it could be a substrate for the seizure prone state. Further studies are needed to clarify the role and neuroanatomic location of both normal and abnormal receptors involved in the regulation of convulsion susceptibility and intensity in the GEPR.

Comparison of data from receptor binding assays between control rats and GEPR brains[1]

Radioligands	K_d (pM) Control	GEPR	B_{max} (fmoles/mg protein) Control	GEPR
H^3-WB-4101	497.0 ± 52.3	509.6 ± 36.2	54.3 ± 2.8	56.0 ± 4.2
H^3-Dihydroalprenolol	1951.7 ± 126.2	3420.3[2] ± 50.9	41.9 ± 2.2	42.7 ± 6.1

[1] K_d- and B_{max}-values were obtained from Scatchard analysis. Each value represents the mean ± SEM of the data from at least 9 animals. [2] Different from corresponding control, $p > 0.02$.

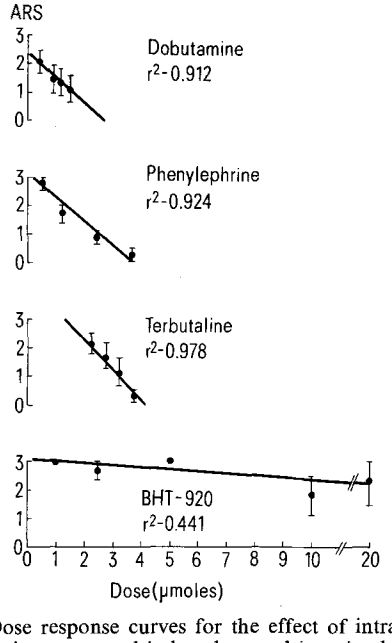

Figure 2. Dose response curves for the effect of intracerebroventricular agonists on sound-induced convulsions in the GEPR. In each case, drug was administered 30 min before testing for convulsion intensity. Each point represents the mean ± SEM for 4–6 animals. The calculated correlation coefficients for each agonist are shown in the figure.

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Cardiac hypertrophy in surgically denervated dogs with aortic stenosis

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Summary. Left ventricular cell hypertrophy in dogs with aortic stenosis was accelerated by surgical denervation of the left ventricle. We conclude that there are neural mechanisms which, when present, inhibit cardiac cell hypertrophy.

We have reported previously that cardiac cell hypertrophy is accelerated by left cervical sympathetic denervation in spontaneously hypertensive rats[2]. In that study, however, the possibility existed that a genetic factor could have participated in the acceleration of the cardiac cell hypertrophy. The present study was designed to exclude the genetic factor, and to employ the pressure factor alone in the production of cardiac cell hypertrophy.

Materials and methods. 18 adult mongrel dogs (12–17 kg) were anesthetized by sodium pentobarbital (30 mg/kg), and artificially ventilated. A left thoracotomy was performed through the 4th intercostal space. The ascending aorta was carefully separated from the main pulmonary artery at a distance 1 cm from the aortic valve and stenosed with a polyethylene band 4 mm wide, monitoring pressure by a catheter (NIH; 7F). The pressure gradient across the aortic valve after aortic stenosis ranged from 30 to 55 mm Hg.

After the operation of aortic stenosis, 10 of the dogs underwent surgical denervation of the left ventricle, using the technique of Geis et al.[3]. The remaining group was sham operated. Surgical denervation of the left ventricle was confirmed by the absence of elevation of the left

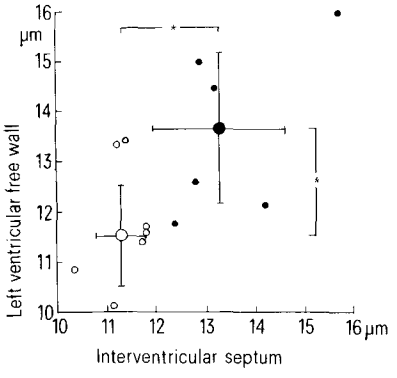

Figure 1. Cell diameters of the left ventricular free wall and the interventricular septum in the denervated group (●) and the sham-operated group (○), which are measured in cross sections at the level of the nuclei. The cell diameters in the denervated group are significantly larger than those in the sham-operated group. Each small circle indicates the mean diameter of 50–60 cells in each dog. Large circles show the mean values for 7 denervated dogs (●) and 6 sham-operated dogs (○). Mean ± SD are shown. * $p < 0.05$.