Effect of Beta-Adrenergic Receptor Blockade on Racing Performance of Greyhounds with Normal and with Denervated Hearts

By David E. Donald, David A. Ferguson, and Sidney E. Milburn

ABSTRACT
A field study was made of the effect of β-adrenergic receptor blockade (propranolol; K6 592) on the racing performance of six normal greyhounds and three with chronic extrinsic cardiac denervation. Records were made of the time to race a 5/16-mile course before and after blockade; the heart rate was recorded by telemetry. After the administration of the β-adrenergic receptor-blocking agent to the normal greyhound, racing time was slightly increased, and maximal heart rate was slightly decreased. After blockade in the cardiac denervated greyhounds, racing time was prolonged, cardiac acceleration was severely limited, and the animals finished running in a state of collapse. The data indicated that the cardiostimulant action of both sympathetic nerves and circulating catecholamines was necessary for maximal performance. After blockade of one or the other of these mechanisms, racing performance was slightly reduced from normal. Withdrawal of both adjuvants severely limited the performance of maximal exercise.

ADDITIONAL KEY WORDS cardiac denervation maximal exercise propranolol catecholamine blockade racing greyhounds heart rate K6 592

Several studies have shown that after extrinsic cardiac denervation the cardiac response of dogs to stress is still of considerable magnitude. Studies from this laboratory have demonstrated that, up to work levels represented by an oxygen consumption of 90 to 100 ml/min per kg STPD, the ability of the dog with a denervated heart to exercise is unchanged from that of the normal dog, and its cardiac output increases to the same extent (1). Also, studies in racing greyhounds have shown that after extrinsic cardiac denervation the capacity for maximal exercise is little reduced from that recorded prior to denervation (2).

Since the adrenal glands and the autonomic nervous system (other than the extrinsic cardiac nerves) were intact, catecholamines released from these structures into the circulation may have played an important part in the responses of these dogs to exercise. In this regard, studies in man (3, 4) have shown that the concentration of epinephrine and norepinephrine in the blood increases rapidly and exponentially with the work load. Experiments on mongrel dogs with extrinsic cardiac denervation exercising under laboratory conditions before and after β-adrenergic receptor blockade with propranolol (1-isopropylamino-3-[1-naphthyloxy]-2-propanol hydrochloride) indicated that circulating catecholamines might be essential in these animals for the performance of severe work (5). Since it is difficult to motivate dogs to exercise to their maximal level under laboratory conditions, recourse was again made to the racing greyhound, an animal bred, trained, and highly motivated to exercise to a maximum. The study was conducted under field conditions at a training track and

From the Section of Surgical Research, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901. Dr. Milburn practices veterinary medicine in Elk Point, South Dakota.

This investigation was supported in part by U. S. Public Health Service Research Grant HE-6143 from the National Heart Institute.

Accepted for publication December 1, 1967.
involved both normal greyhounds and greyhounds with extrinsic cardiac denervation.

Methods

Male and female greyhounds weighing 26 to 33 kg were used. The first group consisted of six normal dogs. After an appropriate period of training and conditioning, a series of ten tests was conducted at intervals of 2 to 3 days between tests. In these tests the time to race a 5/16-mile course was recorded. The studies were then repeated after $\beta$-adrenergic receptor blockade with propranolol or with Ko 592* (1-[3-methylphenoxy]-3-isopropyramino-propanol hydrochloride) (6). The dogs were given intravenously in doses of 1 or 2 mg/kg of body weight. The dogs were raced 8 to 10 minutes after the drug had been given. These tests were concluded with a final run in which an injection of normal saline was substituted for the $\beta$-adrenergic receptor-blocking drug. In these studies of racing after $\beta$-adrenergic receptor blockade and in the final normal run, the heart rate was recorded with an electrocardiograph within 20 seconds from the end of the race.

Efficacy of $\beta$-Adrenergic Blockade in the Normally Innervated Greyhound.—As soon after the race as the heart rate had returned to the control level, and always within 20 minutes of giving propranolol or Ko 592, an attempt was made to evaluate the efficacy of $\beta$-receptor blockade by the degree of reduction in the tachycardia induced by infusion of isoproterenol hydrochloride (Isumpril) or induced by excitement of the animal. A most potent method of stimulating the dog was to have it stand beside the track and watch other dogs racing. It was difficult to interpret the results of excitement and of the intravenous infusion of isoproterenol as indices of $\beta$-receptor blockade owing to the abrupt and large variations in heart rate consequent on variations in cardiac vagal nerve activity. Under these conditions the control heart rate could vary between 60 and 200 beats/min. However, the infusion of isoproterenol (50 $\mu$g/min) resulted in an average maximal heart rate of 231 beats/min (range 205 to 248). After $\beta$-receptor blockade the same dose of isoproterenol resulted in an average maximal heart rate of 148 (range 112 to 188). Similarly the excitement engendered by the dog’s watching a race caused an average maximal heart rate of 238 beats/min (range 157 to 290) before $\beta$-receptor blockade as compared to an average of 192 (range 158 to 217) after blockade.

The animals were then brought back to the laboratory, and a further series of tests was carried out to evaluate the degree to which these drugs were able to block the effects on the heart of electrical stimulation of the cardiac sympathetic nerves and of circulating catecholamines. The tests included blocking the parasympathetic cardiac nerves in the conscious dog with atropine (0.2 mg/kg) and then assessing the ability of the drugs to reduce or abolish the cardioacceleration induced by the intravenous infusion of isoproterenol or $l$-norepinephrine (Levophed). Similar tests were carried out in two conscious dogs 24 hours after bilateral cervical vagotomy and in four anesthetized dogs after acute bilateral cervical vagotomy. The average heart rate in the conscious quiescent dog after bilateral cervical vagotomy or atropine administration was 190 beats/min (range 180 to 208). The intravenous infusion of isoproterenol (130 $\mu$g/min) resulted in an average increase in heart rate of 8 beats/min (range 0 to 15). In the four anesthetized dogs with acute section of the vagi in the neck, the intravenous infusion of $l$-norepinephrine or isoproterenol caused increases in heart rate which ranged from 100 to 150 beats/min. Treatment with propranolol or Ko 592 (1 mg/kg) largely abolished this response. When $l$-norepinephrine or isoproterenol was again infused, frequently there was no change in heart rate; the maximal response was 10 beats/min.

An average control heart rate of 127 beats/min (range 117 to 135) was obtained in the six anesthetized greyhounds with open chest and artificial respiration. Supramaximal stimulation of the right stellate ganglion (10 volts, 15/sec) resulted in an average heart rate of 260 beats/min (range 238 to 288). Repetition of the stimulation 10 minutes after propranolol administration (1 mg/kg) resulted in accelerations in heart rate of 61, 43, 34, 33, 29, and 6 beats/min. In the two dogs showing accelerations of 61 and 43, the dose of propranolol was increased to 2 mg/kg. Repetition of the stimulation resulted in accelerations in heart rate of 45 and 36 beats/min.

The second group of dogs consisted of three females in which extrinsic cardiac denervation had been carried out by the method of Cooper and colleagues (7). No racing history or race times were available prior to cardiac denervation. After recovery from the surgical procedure, the dogs were put into training, and a series of control times of racing over the 5/16-mile course was obtained. These field studies were then repeated after $\beta$-adrenergic receptor blockade with propranolol (1 mg/kg). For comparison with the previous group of normal animals, a normal grey-
TABLE 1
Normal Greyhounds: Time to Race 5/16 Mile before and after β-Adrenergic Receptor Blockade

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight (kg)</th>
<th>Control (10 races)</th>
<th>Propranolol</th>
<th>Ko 592</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.0</td>
<td>32.9</td>
<td>0.4</td>
<td>33.8</td>
<td>Dog lame</td>
</tr>
<tr>
<td>2</td>
<td>29.0</td>
<td>32.6</td>
<td>0.5</td>
<td>32.8</td>
<td>32.9</td>
</tr>
<tr>
<td>3</td>
<td>32.0</td>
<td>32.0</td>
<td>0.5</td>
<td>33.0</td>
<td>33.9</td>
</tr>
<tr>
<td>4</td>
<td>31.4</td>
<td>31.9</td>
<td>0.3</td>
<td>32.6</td>
<td>32.3</td>
</tr>
<tr>
<td>5</td>
<td>32.3</td>
<td>33.6</td>
<td>0.6</td>
<td>35.4</td>
<td>34.1</td>
</tr>
<tr>
<td>6</td>
<td>30.5</td>
<td>31.5</td>
<td>0.3</td>
<td>32.4</td>
<td>32.4*</td>
</tr>
</tbody>
</table>

*Drug given in dose of 2 mg/kg.

The dog was included in this part of the study. Heart rate was recorded by telemetry, and records were obtained on each dog during two normal races and two races after β-adrenergic receptor blockade. As a check on the persistence of cardiac denervation on completion of these field studies, tyramine (60 μg/kg) and atropine (0.2 mg/kg) were administered intravenously, and the effect on heart rate was observed. Neither of these drugs resulted in a change in heart rate greater than 3 beats/min in the dogs with denervated hearts. There was also no immediate change in heart rate when the dogs were suddenly startled or excited.

The results of these experiments are presented in Table 2.

TABLE 2
Heart Rate (Beats/Min) after Racing 5/16 Mile in Normal State and after β-Adrenergic Receptor Blockade in Six Normal Greyhounds

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Propranolol</th>
<th>Ko 592</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of dogs</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Average</td>
<td>282</td>
<td>270</td>
<td>221</td>
</tr>
<tr>
<td>Range</td>
<td>246-304</td>
<td>224-300</td>
<td>206-232</td>
</tr>
<tr>
<td>Time of record†</td>
<td>10</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

*Race after injection of saline.
†Average number of seconds from cessation of running to obtaining first record of heart rate.
variation in the condition of the track contributed to the differences in racing times. Races 1 through 4 were run on successive days, and the condition of the race track was judged to be constant. The effect of the β-receptor-blocking agents was therefore assessed by paired comparisons of the racing time after the administration of the drugs and after the injection of saline. In dog 1 the slowest of the control race times was used for the comparison since the dog became lame during race 3. By use of Student's t-test with paired observations (8), the P values for the separate comparison of races 1, 2, and 3 with race 4 were 0.05 < P < 0.02, 0.2 < P < 0.1, and 0.05 < P < 0.02, respectively.

In each of the six dogs the heart rate was measured before the race and again when the animal had been caught and held steady shortly after the race had ended. The average and range of the heart rates recorded at these times for the normal races (five dogs) and after β-adrenergic receptor blockade are given in Table 2. The lower value for heart rate in the group blocked with KÖ 592 (five dogs) is due to the longer time lapse between the stopping of runs and the first recording of the heart rate. If allowance is made for this delay, there would seem to be little difference in the heart rates recorded after the normal races and those recorded after β-adrenergic receptor blockade with either drug.

Greyhounds With Denervated Hearts.—During the time in which control data were being obtained in these dogs, wet weather rendered the state of the track extremely variable, and only on five of the test occasions were running conditions comparable. The control data for these three dogs, together with the racing times after β-adrenergic receptor blockade, are given in Table 3. Data from a single test (normal and after β-receptor blockade) in a normal greyhound are included for comparison. The behavior of the normal greyhound was similar to that seen in the previous group of six dogs: the racing time after β-adrenergic receptor blockade was slightly prolonged. The behavior of the dogs with cardiac denervation was dramatically different, but not in a fashion best illustrated by the prolongation in racing time. After β-adrenergic receptor blockade, the dogs ran the first half or two thirds of the course in a manner which differed little from that in the control runs, but in the final portion they slowed almost to a walk (Table 4). More impressive was the exhausted condition of the dogs on finishing the course. They sat or lay down

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight (kg)</th>
<th>Five initial control times</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28.0</td>
<td>35.8</td>
<td>0.5</td>
<td>42.4</td>
<td>35.8</td>
<td>39.9</td>
</tr>
<tr>
<td>B</td>
<td>27.0</td>
<td>35.1</td>
<td>0.5</td>
<td>40.5</td>
<td>35.8</td>
<td>38.4</td>
</tr>
<tr>
<td>C</td>
<td>26.0</td>
<td>35.8</td>
<td>0.6</td>
<td>41.3</td>
<td>35.4</td>
<td>40.1</td>
</tr>
<tr>
<td>D</td>
<td>30.0</td>
<td></td>
<td></td>
<td>32.6</td>
<td>34.5</td>
<td></td>
</tr>
</tbody>
</table>

*A, B, and C = greyhounds with denervated hearts; D = normal greyhound.
†Order in which tests were run. Races 1 and 3 after propranolol administration (1 mg/kg).

Greyhounds with Denervated Hearts: Seconds Required to Race 5/16 Mile before and after β-Adrenergic Receptor Blockade

<table>
<thead>
<tr>
<th>Dog</th>
<th>State</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>10.9</td>
<td>11.4</td>
<td>13.5</td>
<td>35.8</td>
</tr>
<tr>
<td></td>
<td>Blockade</td>
<td>11.1</td>
<td>11.6</td>
<td>17.2</td>
<td>39.9</td>
</tr>
<tr>
<td>B</td>
<td>Control</td>
<td>11.1</td>
<td>10.9</td>
<td>12.7</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>Blockade</td>
<td>11.1</td>
<td>11.2</td>
<td>16.1</td>
<td>38.4</td>
</tr>
<tr>
<td>C</td>
<td>Control</td>
<td>11.7</td>
<td>11.8</td>
<td>12.7</td>
<td>38.2</td>
</tr>
<tr>
<td></td>
<td>Blockade</td>
<td>11.4</td>
<td>11.4</td>
<td>17.3</td>
<td>40.1</td>
</tr>
<tr>
<td>D</td>
<td>Control</td>
<td>10.6</td>
<td>10.6</td>
<td>11.4</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>Blockade</td>
<td>11.0</td>
<td>10.9</td>
<td>12.7</td>
<td>34.5</td>
</tr>
</tbody>
</table>

*A, B, and C = dogs with denervated hearts; D = normal dog.
on the track. The tongue and buccal mucosa were cold and ash gray, the femoral pulse was imperceptible, the mouth was widely open, and respiration was deep and gasping. At this moment the animals generally were unresponsive to stimuli. The contrast between this picture and the vigorous condition of the dog at the completion of the race in the control situation was indeed striking. After 7 to 10 minutes, color returned to tongue and mucosa, a femoral pulse was easily felt, and respiration became of a panting rather than a gasping character. Recovery, therefore, was rapid and complete, and the dogs could be returned to their kennel seemingly undisturbed by the experience.

The heart rate as recorded by the tele-metered electrocardiogram was also very different in the two situations. In Figure 1 are shown data before and after β-adrenergic receptor blockade from the three dogs with cardiac denervation together with observations on the normal animal. In the control state in the dogs with denervated hearts, the heart rate rose slowly during the race but was between 180 and 200 beats/min at the halfway mark and reached about 250/min in the final stages of the race. It remained at this value for about 2 minutes after the dog had stopped running, then slowly declined. In contrast, after β-adrenergic receptor blockade, the maximal heart rate did not exceed 180 beats/min and was generally not much above 160, about which value it remained until beyond the fifth minute of recovery. In the normal dog the rate of rise and the maximal heart rate obtained resembled the observations from the control races in the dogs with cardiac denervation. After the dog had stopped running, however, the return toward pre-exercise values was much more rapid than in the denervated animals. In the control race in the normal dog, the rate of rise and the maximal heart rate were greater than after β-adrenergic receptor blockade. In both situations the return of
the heart rate toward pre-exercise levels was equally rapid. On the other occasion in which heart rate was recorded during racing before and after β-adrenergic receptor blockade, the changes were identical with those shown in Figure 1. In the control situation the maximal heart rates on completion of the racing were 231, 250, and 224 beats/min in the three dogs with cardiac denervation. After β-receptor blockade the heart rates at corresponding times were 161, 144, and 155.

Discussion

The present study is concerned with the role of circulating catecholamines in the performance of maximal exercise by dogs with denervated hearts. Implicit in the term "maximal exercise" is the concept of a work load of such intensity that it can be sustained only for a brief period. It is in this respect that the present study differs from previous work in which the exercise was less severe and could be sustained over a period of time (5).

Studies in this laboratory have shown the dog with extrinsic cardiac denervation to be capable of exercising up to levels of oxygen consumption six to eight times that in the resting state and that this capacity for exercise was not altered by bilateral adrenalectomy (1). Also, when induced to exercise to the highest level that could be attained under laboratory conditions, the dog with cardiac denervation performed almost as well after β-adrenergic receptor blockade as before, though failure to complete the exercise test after blockade was observed on a significant number of occasions (5). Refusal to run occurred always in the final moments of exercise at the highest work level. To investigate this point more fully required assurance that the work load was the maximum that the animal could undertake. The suitability of the greyhound for such studies lies in its training and motivation to race. As was demonstrated in the present studies, the drive to race was so intense that the animal would continue running to the point of collapse.

Essential to an interpretation of these data is a knowledge of the degree to which the cardiac β-adrenergic receptors have been blocked either by propranolol or by Ko 592. Both of these agents are competitive antagonists, and their inhibitory effect is inversely related to the concentration of agonist (9). The data indicated that propranolol or Ko 592 in a dose of 1 mg/kg would prevent completely, or nearly so, the effects on the heart of β-norepinephrine or isoproterenol in blood concentrations which resulted in an acceleration in heart rate similar to that occurring in maximal exercise. However, neither blocking agent was as effective in preventing the cardiac excitatory effects of supramaximal electrical stimulation of the cardiac sympathetic nerves, even when the dose was increased to 2 mg/kg. In normal greyhounds the excitement of watching other dogs racing resulted in an average heart rate of 238 beats/min. After treatment with propranolol or Ko 592, similar stimulation produced a tachycardia which averaged 192 beats/min. Since this tachycardia reached its maximal value within 6 to 10 seconds of stimulation, it was presumably due to reflex stimulation of the cardiac sympathetic nerves. Thus in the greyhound, although the effects of circulating catecholamines on the heart practically could be abolished, the blocking agents could produce only a partial blockade of the effects of reflex stimulation of the cardiac sympathetic nerves.

Although the evidence is indirect, being dependent on the drugs' specifically blocking the action of catecholamines on the heart, the experiments suggested that the greyhound with extrinsic cardiac denervation depended on the cardiac action of circulating epinephrine and norepinephrine for the performance of maximal exercise. The prolongation of the racing time in the last third of the course, the limited ability of the heart to increase in rate, and the exhausted condition of the animal on termination of running all emphasize the importance of these blood-borne agents in enabling the heart to meet the demands of maximal exercise. An additional
factor which may be of importance in this regard is the supersensitivity to \( l \)-norepinephrine that is exhibited by the heart some days after resection of the cardiac sympathetic nerves \((10, 11)\). The sustained speed of running in the first 20 seconds of the race after treatment with propranolol probably reflects the ability of the greyhound to briefly support intense muscular activity despite an inadequate flow of oxygenated blood to the active areas. Continued exertion, however, quickly resulted in a marked slowing in speed until finally the animal was forced to stop. Thus, although the heart possesses intrinsic mechanisms which are adequate for the performance of work loads of the magnitude indicated earlier \((5)\), these mechanisms of themselves can increase cardiac output only to a limited extent. Without the cardio-stimulant action of circulating catecholamines, the greyhound with a denervated heart has a severe reduction in its maximal racing speed.

The studies also are of interest in regard to the effect of \( \beta \)-adrenergic receptor blockade on the performance capabilities of the greyhound with a normally innervated heart. Although the racing times were slightly increased after treatment with the drugs, the increase was of borderline significance, and the animals ran well and finished the race in a vigorous manner. The experiments that tested the efficacy with which the \( \beta \)-adrenergic receptors had been blocked suggested it was likely that the effect on the heart of circulating catecholamines had largely been annulled, but that there was only partial blockade of the response to excitation of the cardiac sympathetic nerves. As a result, there was little reduction in maximal heart rate and in the capacity to race at maximal speed. From a study of the effect of \( \beta \)-adrenergic blockade on the cardiac response to maximal exercise in man, Epstein and co-workers \((12)\) concluded that although sympathetic stimulation of the heart contributed to the response to exercise, its importance in increasing the cardiac output was relatively small. In these studies if the motivation to exercise was high and stimulation of the cardiac sympathetic nerves correspondingly intense, then, as in the greyhound, blockade of endogenous nerve stimuli might be relatively ineffective. Then the level of work achieved after the use of propranolol would reflect the effects of impairment of sympathetic nerve stimulation of the heart and not the effects of its complete absence.

Our previous studies \((1, 2, 5)\) on the performance of maximal and submaximal work in dogs and this present study perhaps put in proper perspective the role of the cardiac sympathetic nerves and of circulating catecholamines in the ability to exercise. Both sympathetic nerve activity and the stimulant action of circulating epinephrine and norepinephrine seem necessary for the performance of maximal exercise. The absence of one or the other of these mechanisms little reduces the capacity for maximal work, this being defined as the most severe exercise of which the animal is capable, and which of necessity cannot be sustained for any length of time. The degree of reduction is perhaps greater when the action of the sympathetic nerves on the heart, rather than that of the blood-borne catecholamines, is eliminated. When both adjuvants are withdrawn, and the heart must increase its power of contraction solely through the Frank-Starling mechanism of greater fiber length, then there is a very considerable reduction in the capacity for maximal exercise although the animal is by no means a "cardiac cripple."

Acknowledgments

The greyhounds were trained by Mr. and Mrs. W. Barret, Jefferson, S. D., whose enthusiasm and generous help with these studies the authors acknowledge.

References


Effect of Beta-Adrenergic Receptor Blockade on Racing Performance of Greyhounds with Normal and with Denervated Hearts
DAVID E. DONALD, DAVID A. FERGUSON and SIDNEY E. MILBURN

Circ Res. 1968;22:127-134
doi: 10.1161/01.RES.22.2.127

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1968 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/22/2/127

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/