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Training-induced Bradycardia and Intrinsic Heart Rate in Rats

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Summary. After 10 weeks of treadmill training, female Sprague-Dawley rats had developed a bradycardia at exercise on submaximal work loads. This bradycardia was also present after autonomic denervation and in isolated perfused heart preparations. The heart weight/body weight ratio was increased in these trained animals compared to untrained littermates. Sympathectomized, trained rats developed the same degree of cardiac hypertrophy, but their heart rate after denervation and in the isolated heart was the same as in sympathectomized, untrained rats.

It is concluded that the bradycardia of trained and thereafter denervated animals seen in this and a previous investigation represents an adaptation within the heart itself, since it was present in the isolated heart. These results thus provide further evidence for a non-neural component in training-induced bradycardia. Since the trained sympathectomized rats had a cardiac hypertrophy but no reduction of intrinsic heart rate, it seems likely that the myocardial mass is of minor importance for the level of intrinsic heart rate.

Key words: Physical training – Bradycardia – Intrinsic heart rate – Cardiac hypertrophy – 6-hydroxydopamine

One of the circulatory adaptations characteristic of the physically well trained individual is a bradycardia at rest and during exercise at submaximal work loads (Åstrand and Rodahl 1977). Traditionally, this heart rate adaptation has been attributed to changes of heart rate regulation by the autonomic nervous system. In more recent experiments, however, it has been found that trained individuals exhibit a bradycardia also after abolition of the autonomic nervous influence (Bolter et al. 1973; Hughson et al. 1977; Lewis et al. 1980; Smith and El-Hage 1978; Sutton et al. 1967) which indicates that the intrinsic heart rate is also

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altered by training. The concept "intrinsic heart rate" (IHR) was originally coined by Jose (1966) to denote the heart rate in man after full pharmacological autonomic blockade, and we have adopted the term for the heart rates of animals depleted of autonomic nervous influence on the heart.

This is accomplished by subjecting rats to 6-hydroxy-dopamine (6-OH-DA), which causes a long lasting degeneration of the adrenergic nerves (Malmfors and Thoenen 1971), vagotomy and spinal cord destruction (pithing). We have earlier shown that rats trained by treadmill running and thereafter denervated had a lower heart rate than untrained animals treated in the same way (Sigvardsson et al. 1977). Sympathectomized and thereafter trained rats did not develop any reduction of IHR and therefore we concluded that an intact adrenergic nervous system is essential for development of training-induced IHR decrease.

Advantages with the type of denervation used are, that compared to pharmacological blockade with atropine and beta receptor antagonists, less doubt can be raised about the completeness of denervation, and that despite the denervation, the heart is intact, unaffected by surgical intervention and remaining in the circulatory system of the body. On the other hand, with this denervation in situ the heart rate can theoretically be influenced by circulating metabolites or transmittor substances or mechanical factors such as venous return or afterload.

In order to exclude that such extracardiac factors were influencing the results obtained with the above described preparations, the present study was undertaken to investigate whether the heart rate is lower in trained than in untrained animals also in an isolated heart preparation. The heart rates of rats sympathectomized before the training period were also measured in isolated hearts.

Methods

Animals

Thirty-one female Sprague-Dawley rats, littermates, were treated with 6-hydroxydopamine (6-OH-DA), in doses of 50 mg/kg body weight every week from the age of 2 months. Another 30 female rats from the same litters as the 6-OH-DA treated ones served as untreated controls. From each group 15 rats were randomly selected for training from the age of 3 months.

Training and Exercise Testing

Before the start of the training program, three small silver electrodes for ECG-recordings during exercise, were implanted subcutaneously on the back of each rat (Ekblom et al. 1973). The rats were exercised on a Quinton rodent treadmill, model 42-15, at a speed of 25–30 m/min for 1 h a day, 5 days a week for 10 weeks. After the training period all animals were exercise tested. The rats then ran for 10 min at a speed of 21 m/min and subsequently for 5 min at 25.5 and 30 m/min respectively at an inclination of about 3°. Heart rate (HR) was recorded in 10-s periods three times during the last 3 min of each work load, when according to pilot studies steady state values are achieved, and the average was used. During 3 days prior to the tests, all rats were exercised for ½ h a day in order to accustom them to treadmill running (Sigvardsson et al. 1977).

Denervation and Heart Rate Recording

After the exercise test, all rats were injected with 6-OH-DA, 50 mg/kg i.v. in order to obtain the same degree of denervation supersensitivity in all animals (see Discussion) and to prevent the spontaneous release of noradrenaline from the nerve endings during the final HR measurements. The animals were anaesthetized 18–24 h after this with aether and subjected to pithing (Shipley and Tilden 1974) and bilateral cutting of the vagus nerves in the cervical region. The animals were artificially ventilated and a catheter was inserted into the carotid artery and connected to a Statham P 23 DC transducer for blood pressure and HR recordings on a Grass Polygraph. After 30 min the heart was rapidly removed and transferred to as modified Langendorff perfusion apparatus (Wennmalm 1979) were it was perfused with tyrode solution, aerated with 6.5% CO_2 in O_2. The perfusion pressure was 60 mm Hg and the temperature 35° C, as the heart was more liable to arrythmias at higher temperatures. The apex of the heart was connected to a strain gauge transducer and HR was recorded on the Polygraph. In a few hearts (1–2 in each group) arrythmia occured before the end of the experiment. They were then excluded from further HR measurements. Immediately after completion of the HR measurements, the hearts were blotted and weighed.

Statistical Methods

For statistical comparisons, Student's *t*-test for independent sample means has been used, and statistically significant differences will be referred to as "significant" in the text ($p < 0.05$ unless otherwise stated). Correlations between heart weights and heart rates were calculated by linear regression.

Results

When the animals were tested by exercise at the end of the experiment, training was associated with a lower HR, by 20–26 beats/min (difference of mean values at the three work loads) in the untreated ("normal") group. The trained (T) animals treated with 6-OH-DA ("sympathectomized") also showed a lower HR during exercise (by 14–26 beats/min) than the untrained (UT) animals. Treatment with 6-OH-DA resulted in higher heart rates than were found in the normal animals so that the HR of the T sympathectomized animals were similar to those of the UT normal animals.

After pithing, there remained a significant HR difference of 27–37 beats/min between T and UT normal animals (Fig. 2), but there was no HR difference between T and UT sympathectomized rats after pithing (Fig. 3). The situation was the same regarding the isolated hearts, significantly lower HR of T than UT normal rats (38–53 beats/min) (Fig. 2) and no significant difference between the sympathectomized groups (Fig. 3).

Pre-training body weights were lower in the two sympathectomized groups than in the two normal groups combined (211 g vs. 231 g, $p < 0.001$). After the training period there was no significant body weight difference between T and UT animals, neither normal nor sympathectomized and the pre-training difference between normal and sympathectomized had disappeared (Table 1). The average absolute heart weights were of the same magnitude in UT normal

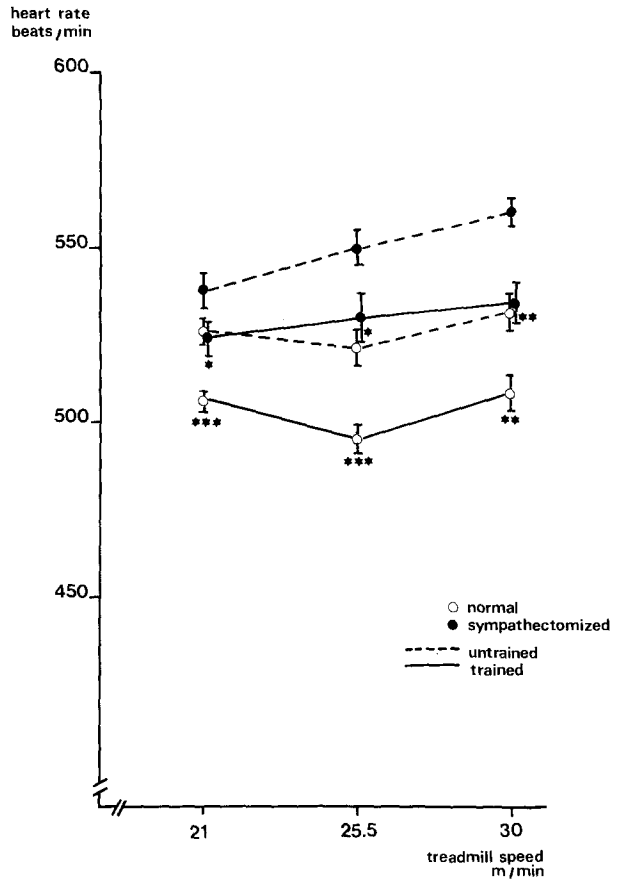
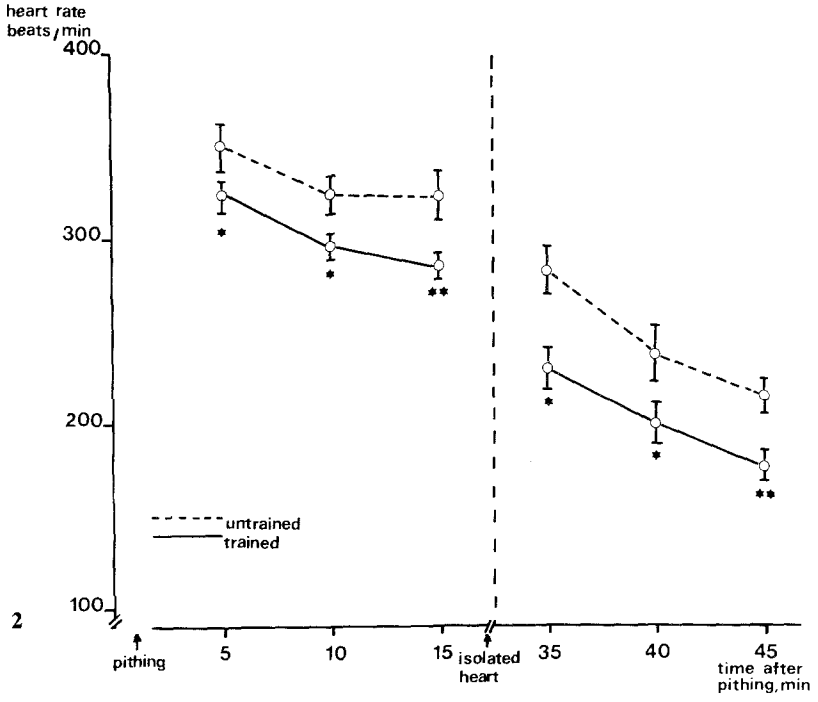


Fig. 1. Heart rates during exercise at three different work loads in trained and untrained, normal and sympathectomized rats, mean values ± SEM. Asterisks denote statistically significant differences compared to corresponding untrained group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

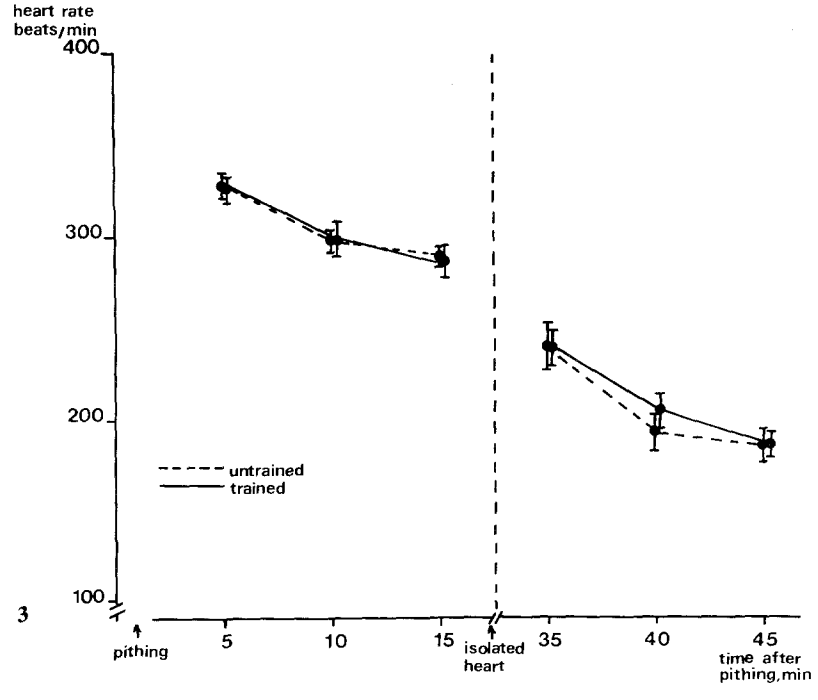
and sympathectomized (1.172 and 1.180 g) (Table 1). The difference between T and UT was statistically significant in the sympathectomized animals. The heart weight/body weight (HW/BW) ratio was significantly greater in T than in UT animals, both normal and sympathectomized (Fig. 4). No significant correlation was found between IHR or HR of the isolated heart on one hand and heart weight or HW/BW ratio on the other, neither in normal, in sympathectomized, nor in all groups combined.

Fig. 2. Heart rates in normal trained and untrained rats after denervation (6-OH-DA, vagotomy and pithing) and in the isolated heart, mean values ± SEM. Symbols as in Fig. 1

Fig. 3. Heart rates in sympathectomized trained and untrained rats, after denervation and in the isolated heart, mean values ± SEM. Symbols as in Fig. 1


heart rate
beats/min
400
300
200
100
untrained
trained
pithing
5
10
15
isolated
heart
35
40
45
time after
pithing, min

2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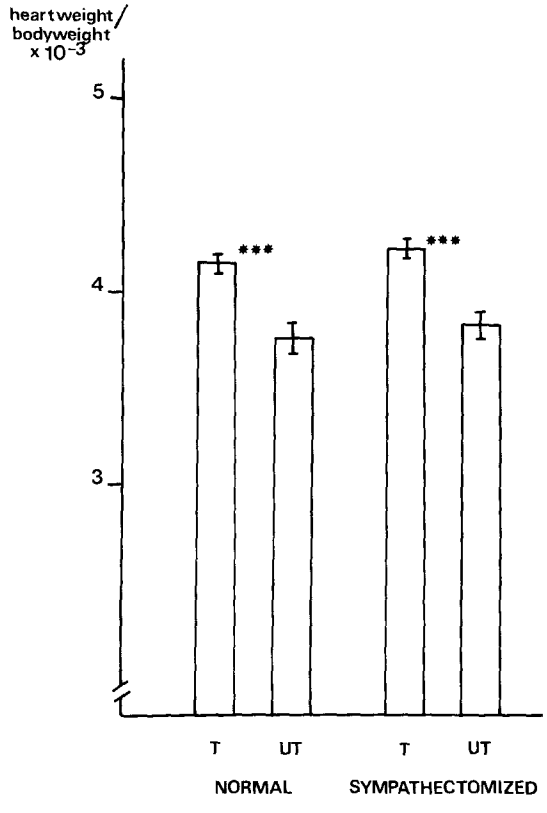

Fig. 4. Heart weight/body weight ratio of trained and untrained sympathectomized and normal rats, mean values ± SEM. T = trained, UT = untrained. *** Statistically significant difference between T and UT, $p < 0.001$

Table 1. Body weights before training and body weights and heart weights after the training period (T = trained, UT = untrained)

		Before training body weight (g)	After training	
			Body weight (g)	Heart weight (g)
Normal UT	m	234	291	1.104
	SEM	4	3	0.027
	n	14	14	10
Normal T	m	227	282	1.172
	SEM	2	5	0.025
	n	15	15	13
SYMP ECT UT	m	217	288	1.100
	SEM	5	4	0.023
	n	16	16	15
SYMP ECT T	m	205	279	1.180[a]
	SEM	3	4	0.015
	n	15	15	15

[a] Significant difference compared to corresponding UT group, $p < 0.01$

Discussion

Mechanisms of Cardiac Hypertrophy

The HW/BW ratio was significantly higher in both T groups than in both the UT ones and not significantly different between the two T groups. The most accurate method to define cardiac hypertrophy has been subject to debate, and the use of the weight of relatively small organs related to body weight has been criticized by Heroux and Gridgeman (1958). Others, however (Jaweed et al. 1974) have found that the weight increase of the heart almost parallels the body weight increase in female normal rats age 16–26 weeks which justifies the use of the heart weight/body weight ratio as a measure of cardiac hypertrophy. This ratio has also been frequently used and HW/BW ratios of T and UT rats in this study are similar to those reported by others (Arcos et al. 1968; Crews and Aldinger 1967; Jaweed et al. 1974; Oscai et al. 1971a, b; van Liere and Northup 1957).

Accordingly, the chemical sympathectomy with 6-OH-DA did not prevent the development of cardiac hypertrophy though training. The circulatory effects of this treatment are complex. The heart rate during exercise was higher in 6-OH-DA treated rats than in normal animals in this present and in our previous investigations (Ekblom et al. 1973; Sigvardsson et al. 1977). This can be explained by a denervation supersensitivity, i.e., the denervation prevents the mechanism of inactivation of circulating adrenal catecholamines by neuronal uptake (Finch and Leach 1970) and these catecholamines thus exert a stronger effect at the receptor sites. Previous findings of an increased adrenal weight in 6-OH-DA treated trained rats (Sigvardsson et al. 1977) and an increased adrenal catecholamine turnover after 6-OH-DA (Mueller et al. 1969) also suggest a compensatory increase of the production of adrenal catecholamines to balance the lowered blood pressure and peripheral resistance seen in animals as rest after 6-OH-DA treatment (De Champlain and van Ameringen 1972; Gauthier et al. 1972). Thus the adrenergic stimulation on the heart or at least its "net effect" seems to be increased after 6-OH-DA treatment.

Also the blood pressure during exercise may be increased since the blood pressure reaction to i.v. noradrenaline is enhanced after 6-OH-DA (Krakoff and Ginsburg 1973). Although the adrenal catecholamines in rats are mainly in the form of adrenaline (von Euler 1956) it has been shown that after sympathectomy the compensatory increased production of noradrenaline is much greater than the adrenaline production (Östman-Smith 1976). It is therefore conceivable that the myocardial work expressed as the product of heart rate and blood pressure (Gerola et al. 1957; Robinson 1967) is increased during exercise in 6-OH-DA treated rats.

Another way of studying the role of the adrenergic nervous system in the adaptation to training, beta adrenoceptor blockade, was used in an investigation of a similar design (Nylander 1981a). When rats chronically treated with the cardioselective beta receptor antagonist metoprolol were trained, they got a lower HW/BW ratio than groups of untreated rats. The results are in accordance with findings in trained propranolol treated rats by Harri and Narvola (1979)

who also conclude that the adrenergic stimulation seems to be essential for the development of cardiac hypertrophy. Since heart rate and blood pressure during exercise both are lowered by beta adrenergic blockade (Conolly et al. 1976) cardiac work during exercise is then decreased, in contrast to the effect of 6-OH-DA treatment. These results therefore support the hypothesis that the magnitude of the cardiac work could be one factor of importance for the development of cardiac hypertrophy through training. This is denied by Östman-Smith (1976) who trained rats after guanethidine treatment and reported that this type of sympathectomy abolished the training-induced cardiac hypertrophy. She concludes that the cardiac sympathetic nerves release a substance which is necessary for the induction of the hypertrophy. However, that type of sympathectomy caused a retardation of body growth, which makes it difficult to compare the heart weights of sympathectomized and untreated animals. It is also uncertain if the work load in relation to body weight during training (swimming) was the same in sympathectomized and untreated rats. We have noted in unpublished experiments that rats frequently exhibit a bradycardia during swimming, probably due to the "diving reflex". As Östman-Smith does not report the heart rate during exercise or after the training period, it is difficult to estimate the myocardial work in guanethidine sympathectomized and untreated animals during swimming. Accordingly, the different training methods as such and the different methods of sympathectomy could explain the discrepancy between our and her results.

Mechanisms of Training-induced Bradycardia

The efficiency of our training program is documented by the significantly lower HR in T than in UT normal animals at all levels of exercise (for detailed methodological discussion, see Nylander 1981b). In the sympathectomized animals the HR difference between T and UT was also significant, but the exercise HR of these two groups were higher than in the normal groups, respectively. This is in accordance with our previous findings (Sigvardsson et al. 1977) where the exercise HR of sympathectomized UT rats increased progressively during the training period, but remained constant in the T group. The similar post training results in the two investigations makes it conceivable that the same mechanism was responsible for the results of this and the previous investigation. Although the exercise HR difference between T and UT sympathectomizd rats must be interpreted as an effect of training, it is different from that in normal animals. This different HR adaptation is further supported by the IHR results, with a significantly lower HR after denervation in T than in UT normal rats, but no difference between the sympathectomized groups.

In this study hearts from rats with a proven relative bradycardia during exercise and after pithing and denervation were subsequently used in isolated heart preparations. In this case the HR difference between T and UT normal rats was still present. These findings indicate that the IHR decrease found in trained and thereafter 6-OH-DA treated, vagotomized and pithed rats in this and in our

previous investigation (Sigvardsson et al. 1977) really represents a change within the heart itself and could not be explained solely by extracardiac factors.

Two other investigators (Östman-Smith 1979; Tipton et al. 1977) using Langendorff preparations have not found any HR difference between hearts of trained and sedentary rats. However, in none of those investigations a training-induced exercise bradycardia in the intact animal was documented.

Accordingly, training in sympathectomized animals caused a cardiac hypertrophy but no decrease of the IHR compared to UT sympathectomized animals. This indicates that an increased myocardial mass is not necessarily accompanied by a reduction of IHR. In intact animals and in humans (Lewis et al. 1980) larger hearts and lower IHR have been seen simultaneously in trained individuals, but whether there is a causal relationship remains to be proven. The lack of correlation between cardiac mass and IHR in normal rats in this study also rather speaks against a direct relationship. It has been proposed that a repeated increase of the diastolic filling during exercise and thus "stretching" of the atria (Frick et al. 1967) alters the function of the sinus node thereby causing a bradycardia. This could explain both the absence of IHR reduction despite a cardiac hypertrophy in sympathectomized rats in this study (where the heart rate during exercise, and probably also at rest, was high, which diminishes the diastolic filling) and the bradycardia without a concomittant cardiac hypertrophy after training with beta adrenergic blockade (Nylander 1981a) since an atrial dilatation would not alter the heart weight substantially.

The mechanisms maintaining the established training-induced bradycardia and IHR reduction may not be the same as those responsible for their development. The fact that animals trained after sympathectomy in this and the previous study did not get a lower IHR than the untrained ones, indicates that adrenergic factors are involved in the latter process.

Thus the following hypothesis concerning the development and maintenance of training-induced bradycardia is suggested: During the period of regular exercise the increase in sympathetic activity induces a lowering of exercise and intrinsic heart rate. Once the lowering of intrinsic heart rate has been established through training the effect is maintained without further cardiac autonomic activity, through factors within the heart itself.

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