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Influence of long-term beta receptor stimulation with prenalterol on intrinsic heart rate in rats

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Summary. Previous studies have shown that the intrinsic heart rate (IHR) may undergo changes, e.g., decrease after long-term endurance training. The mechanism for this adaptation is not known. In this study, rats were subjected to long-term oral treatment with the beta receptor stimulating drug prenalterol. During the treatment period heart rates at rest and during submaximal exercise were measured. Heart rate after 30 min rest and also 2 min after exercise was higher in the treated animals, due to the beta stimulation. The treated rats had a significantly lower heart rate increase during exercise than untreated controls, consistent with a partial beta-blocking effect of the drug in states with a high endogenous sympathetic activity. Therefore, the animals were not trained but only exposed to the increased stimulation of cardiac beta receptors accomplished by the drug while at rest. After 25 weeks, prenalterol was withdrawn and the IHR was measured in situ after a denervation procedure. The treatment with prenalterol had not altered the IHR. Our previous results from training studies indicate that a heart rate increase above a certain level or the stimulation of cardiac beta receptors are not the main stimuli for a lower setting of the IHR as seen after endurance training. In this study chronic beta receptor stimulation with prenalterol did not influence the IHR, which supports that hypothesis.

Key words: Intrinsic heart rate – Beta receptor stimulation – Prenalterol – Exercise heart rate

Introduction

The heart rate (HR) of an individual is determined by the inherent rate of the cardiac pacemaker (intrinsic heart rate, IHR) and the activity of the autonomic nervous system. The short-time variations of HR, e.g., in response to variations in physical activity during the day, are mainly accomplished by alterations in autonomic nervous activity. On the other hand, an alteration in the IHR has been shown, e.g., a decrease as a part of the cardiovascular adaptation to endurance training both in humans (Frick et al. 1967; Lewis et al. 1980) and in animals (Bolter et al. 1973; Hughson et al. 1977; Nylander et al. 1982). It is conceivable that this change of IHR has relevance for the actual HR setting, since the magnitude of the difference between endurance athletes and sedentary men was the same for both resting HR, HR during exercise at submaximal and maximal work loads and IHR (Lewis et al. 1980). Accordingly, a decrease of IHR could explain the downward displacement of the HR level at rest and during exercise in very well-trained men. Also, it has been reported that ageing of rats is associated with a lowering of IHR of the same magnitude as the decrease in maximal HR with age (Corre et al. 1976). For the understanding of both the longitudinal effects of physical training and cardiac disorders with a pathological bradycardia, it may therefore be of interest to investigate the potential variations of IHR.

The mechanisms for the adaptation of IHR are not known at present. We have previously shown that animals trained after chemical sympathectomy with 6-hydroxy-dopamine (6-OH-DA) did not develop a reduction of IHR, in contrast to untreated rats subjected to the same training program (Nylander et al. 1982). In fact, this denervation resulted in an increased sympathetic influence on the heart, because of a compensatory increase in adrenal activity in combination with denervation supersensitivity. It was therefore concluded that the stimulation of cardiac beta receptors or the increase of HR above a certain level during exercise are not the major stimuli to a decrease of IHR.

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However, the denervation with 6-OH-DA has complex effects, e.g., lowering of blood pressure and peripheral resistance (De Champlain and van Ameringen 1972; Gauthier et al. 1972). In trying to elucidate more specifically the role of increased sympathetic activity on the heart it was therefore necessary to find another method. In this study, with the main objective of investigating the effect on IHR of chronic stimulation of cardiac beta receptors, we used long-time treatment of rats with prenalterol. Prenalterol is a beta receptor agonist that binds to both beta-1 and beta-2 receptors but exerts a stimulation mainly on beta-1 receptors (Mattson et al. 1982). It stimulates contractility more than HR when compared to beta-2 and nonselective beta receptor agonists, but it also has a positive chronotropic effect (Manders et al. 1980).

Methods

Fifty-two female Sprague-Dawley rats of equal age and body weight were included in the investigation. Before the study, three small silver electrodes were implanted subcutaneously on the back of each rat for ECG recordings (Nylander et al. 1982). From the age of 8 weeks, 26 of the animals, randomly selected, were fed prenalterol-containing rat pellets, 5 mg · g^{-1} pellet. The 26 control animals were fed pellets with exactly the same constituents except prenalterol. All animals were housed in pairs in the same temperature- and light-controlled room. The administration of prenalterol lasted for 25 weeks, during which time subgroups of rats were subjected to different investigations. The design of the study is outlined in Fig. 1.

Exercise tested group (group A)

To test the effect of prenalterol on HR, this was measured during activity, with an exercise test, and at rest. After 6–8 weeks of prenalterol administration the exercise test was performed in 12 prenalterol treated (P) and 12 control rats (C). A Quinton rodent treadmill, model 42-15, was used. The rats were accustomed to running on the treadmill for 15–20 min at successively increasing speeds up to 25 m · min^{-1} on 3 consecutive days prior to the exercise test. In the test the rats first ran for 10 min at a speed of 14 m · min^{-1} and then for 5 min each at 20.5 and 27 m · min^{-1} respectively. HR was recorded three times during the last 3 min of each work period, and the mean value of these recordings was used in the presentation of the HR value for each work load (Nylander et al. 1982). HR was also recorded 2 min after exercise with the animal sitting on the treadmill. These tests were performed between 8–12 pm. In a pilot study, the average plasma concentration of prenalterol at that time of the day in six rats was 720 nmol · l^{-1} (range 390–1,220). A maximal beta receptor stimulation, measured as HR increase in conscious, ganglion blocked animals, is reported at a plasma concentration of 400 nmol · l^{-1} (Carlsson, Hässle AB, personal communication).

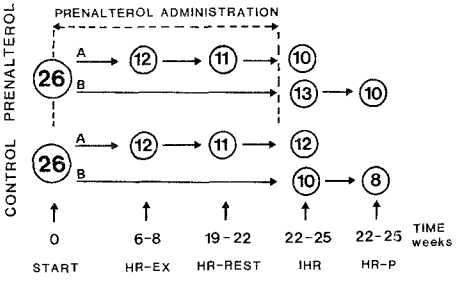

Fig. 1. Schematic design of the study. Figures in circles = number of rats. A = exercise-tested rats, B = non-exercise-tested rats, HR-EX = exercise test, HR-REST = resting HR measurement, IHR = denervation and measurement of intrinsic heart rate, HR-P = measurement of HR after prenalterol iv. For technical reasons, all measurements were not obtained in all animals

Resting HR was measured 19–22 weeks after the beginning of the study in 11 P and 11 C rats, that previously had been exercise tested. These measurements were also performed between 8 and 12 pm. The rats were placed individually in small dark cages and HR was recorded on a Mingograph (Siemens-Elema, Stockholm, Sweden) ink-jet writer with thin cables connected to the subcutaneous electrodes, every 5th min for 30 min.

After 22–25 weeks, the IHR was measured according to a previously described procedure (Nylander et al. 1982) in 10 P and 12 C rats from group A. Three days before the denervation, prenalterol was withdrawn and all rats received control pellets, to avoid drug influence on the final measurements. Body weights were measured just prior to the denervation procedures. 6-hydroxydopamine, 50 mg/kg body weight (bw), was injected iv. (Nadeau et al. 1971). Twenty-four hours after this, the animals were anaesthetized with ether and subjected to pithing (Shipley and Tilden 1947) 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 for blood pressure (BP) and HR recordings. HR and BP were recorded every 5th min for 30 min after pithing. Body temperature was controlled during the measurements, and after their completion an arterial blood sample was taken for blood gas analysis. The heart was then removed and the ventricles carefully dissected free, rinsed in saline, blotted, and weighed.

Acute effect of iv. prenalterol after denervation (group B)

Thirteen P and 10 C rats that had not been exercise tested but otherwise had received the same diet and treatment as the animals in group A, were also pithed and denervated after 22–25 weeks as described above. HR was recorded every 5th min for 30 min. In 10 P and 8 C rats prenalterol, 3 μg · kg^{-1} bw was injected iv. via a catheter in the jugular vein and the HR and blood pressure increase was recorded for 10 min. Blood gases and weights of the cardiac ventricles were determined as above.

Thus, IHR was measured in totally 23 P and 22 C rats from group A and B. Differences between P and C groups were tested for significance with Student's *t*-test for independent sample means. Significant in the text denotes statistical significance, $P < 0.05$ unless otherwise stated. Results in the text are given as mean + SEM.

Results

Heart weight and body weight

Before the study the mean body weight (bw) of the P group was 196 ± 1 g and the body weight of the C group 198 ± 1 g. At the end of the study, it was 306 ±

5 g in P and 302 ± 5 g in C rats. The wet weight of the cardiac ventricles (hw) was 0.874 ± 0.016 g in P and 0.863 ± 0.013 g in C and hw/bw ratio 0.29 ± 0.004% in both P and C animals. None of the weight differences were significant. This holds true also for the subgroups of rats that were exercise tested (A) or only denervated at the end of the study (B). Neither of the subgroups diverged from the whole group of animals with respect to bw or hw.

Heart rate

HR during exercise at the lowest work load was the same in P and C rats. The HR increase during exercise was smaller in the P group, resulting in a significantly lower HR ($P < 0.01$) at the highest work load in this group compared to C rats (Fig. 2). HR measured after 2 min rest immediately after exercise was, in contrast, significantly higher in P than in C animals.

When resting HR was measured during 30 min, 13–14 weeks after the exercise test there was no significant difference between average HR for the whole period between P and C rats (460 ± 7 vs 458 ± 10 beats · min^{-1}). However, the resting HR decreased progressively during the 30 min rest in C animals whilst it remained constant in P rats (Fig. 3). Consequently, the values measured after 30 min rest were significantly higher in P than in C (462 ± 9 vs 436 ± 7 beats · min^{-1}).

Heart rates after denervation (IHR) were measured for 30 min in totally 23 P and 22 C rats (Groups A and B). As shown in Fig. 4 there was no IHR difference between P and C rats. There was a significantly higher average body temperature after

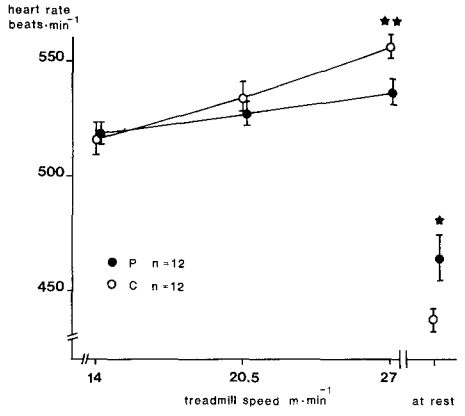

Fig. 2. Heart rate responses to graded exercise in prenalterol treated *(filled circles)* and control rats *(open circles)*, mean values + SEM. Heart rate values 2 min after exercise are also indicated. *: $P < 0.05$ **: $P < 0.01$

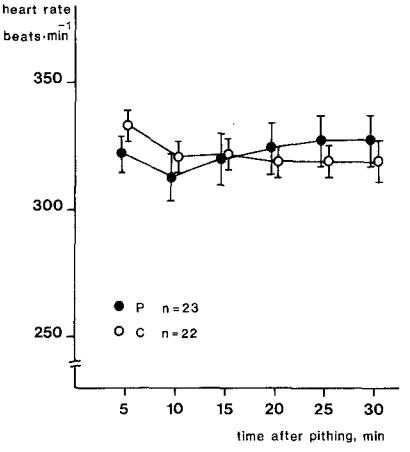

Fig. 4. Heart rates after denervation (IHR) in prenalterol treated and untreated rats. Symbols as in Fig. 3

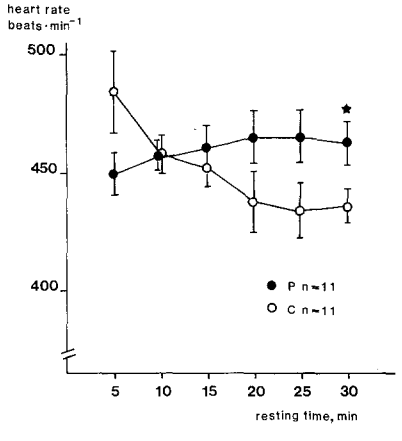

Fig. 3. Resting heart rates during 30 min in prenalterol treated and control rats. Symbols as in Fig. 3

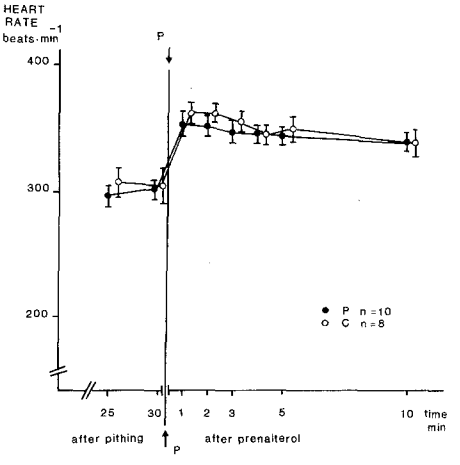

Fig. 5. Heart rates after iv. administration of prenalterol, 3 μg/kg bw *(arrow)* to denervated prenalterol treated and control rats. Symbols as in Fig. 3

40 min in P than in C rats. Otherwise there was no significant difference in the body temperatures or arterial PO_2, PCO_2, BE or Hb measured in the pithed rat preparations. All these variables were within normal limits. Mean blood pressures did not differ significantly at any time after pithing.

Acute effect of iv. prenalterol

When prenalterol was administered iv. to denervated rats, 10 P and 8 C, 30 min after pithing, the average HR increase was similar in P and C (51 ± 7 vs 58 ± 10 beats $\cdot$ min^{-1}) and the HR level reached after injection was also the same in P and C animals (Fig. 5). The average increase of mean BP upon injection was 10 mm Hg in P and 18 mm Hg in C rats. This difference was not significant.

Discussion

In a pilot study the dosage of prenalterol used was shown to give plasma concentrations sufficient for a significant beta receptor stimulation. To document this further we measured HR during exercise and at rest. A beta receptor blocking effect of prenalterol in states with a high endogenous sympathetic activity has previously been suggested in human studies (Hjalmarson et al. 1982; Dahlström et al. 1983). Its properties as a partial agonist have also been demonstrated by Kenakin and Beek (1980). Our P rats had significantly lower HR during exercise of high intensity than C rats, which speaks in favour of such a dual action of prenalterol.

It is difficult to obtain true resting HR in rats because the measurement itself invariably imposes some degree of disturbance and stress. We tried to minimize this by using previously implanted subcutaneous ECG-electrodes, performing the measurements in a quiet room etc. Nevertheless, the HR obtained during the 30 min rest are too high to represent strictly basal conditions.

The average HR in P rats was not higher than in C rats. However, during the 30-min resting period the resting HR of C rats decreased progressively but remained constant in P rats (Fig. 3). At the beginning of the resting HR measurements the endogenous sympathetic tone is likely to be at its highest, making the partial beta receptor blockade appear. As the sympathetic activity decreased during the resting period when the rats got accustomed to the situation, the stimulating properties of the drug would dominate, thereby unmasking the difference between P and C animals, as seen at the end of the resting period. This indicates that the basal HR was higher in P than in C rats. HR immediately after exercise was also higher in P than in C rats, which indicates that periods of dominating beta receptor blockade due to stress or activity are of short duration. Besides the care of the animals, no potentially stressful activities were performed in the room that the rats were housed in during the treatment period. Accordingly, both the plasma concentrations and the resting HR measurements indicate a predominating beta receptor stimulation accomplished by the drug during the treatment period.

The prenalterol treatment had no influence on bw, hw, or hw/bw ratio. This lack of cardiac hypertrophy after chronic beta stimulation differs from results by Harri et al. who reported a significant cardiac hypertrophy after repeated injections of isoprenaline (Harri and Narvola 1979) or noradrenaline (Harri et al. 1982). There are several possible explanations to this divergence. First, the effect may be related to the degree of inotropism. Prenalterol has a maximal effect about 80% of that of isoprenaline (Mattson et al. 1982). Also, during activity of the animals, the effect may be less due to a partial beta receptor antagonism (see above). Secondly, isoprenaline increases the levels of cyclic AMP which could be a factor of importance for cardiac hypertrophy. The stimulating effects of prenalterol are not accompanied by increased cardiac concentrations of cyclic AMP (Hedberg et al. 1982). We have also considered the possibility of development of tachyphylaxis and thus a reduced stimulating effect during the treatment period. The HR increase to the acute iv. administration of prenalterol at the end of the study was, however, the same in P and C rats which rules out this third possibility.

The main object of this study was to investigate whether a long-time stimulation of cardiac beta receptors would alter the intrinsic heart rate. Should that occur it could be one mechanisms for the IHR decrease seen as an effect of physical training. Our hypothesis was, however, that this is not the case. Rats treated with the cardioselective beta receptor antagonist metoprolol during a training period developed a significant relative bradycardia during exercise and a reduction, although not statistically significant, of IHR (Nylander 1981). In another study, rats that were trained after 6-OH-DA treatment that in contrast to metoprolol causes the HR during exercise to be higher than in untreated animals developed no reduction of IHR (Nylander et al. 1982). Both investigations support the theory that the beta receptor stimulation per se or the resulting HR increase are not the primary stimuli for a reduction of IHR (Nylander and Areskog 1982). As proposed also by previous investigators (Frick et al. 1967; Barnard et al. 1976) an atrial dilatation caused by an increased

venous return during repeated exercise may be a factor of importance for the training-induced bradycardia and reduction of IHR. The tachycardia from excess beta stimulation would, because of shorter diastole, not allow sufficient time for this atrial stretching to occur. Harri and Kuusela (1982) found that repeated noradrenaline injections did not alter the intrinsic atrial rate in rats. The results from the present investigation are also in accordance with the above mentioned theory since the selective stimulation of cardiac beta receptors accomplished with oral prenalterol did not alter the IHR in the treated group of rats.

In conclusion, this study has shown that chronic beta receptor stimulation by long-time treatment with prenalterol did not influence the IHR of rats, nor did it cause cardiac hypertrophy. This provides further support to the theory that the lower setting of IHR seen e.g., after endurance training is not accomplished by a repeated beta receptor stimulation.

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