Effect of exercise training on the chronotropic response of isolated rat atria to atropine[1]

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Summary. Atria isolated from rats after 7 weeks of exercise training beat at a slower rate than did atria from sedentary controls. Atria from exercised rats showed a significant acceleration in response to atropine.

A program of endurance training characteristically produces a resting bradycardia. Numerous suggestions have been made regarding the mechanism of this bradycardia, including increased parasympathetic[2] or decreased sympathetic[3] activity to the heart. Bolter et al.[4] reported that isolated atria from exercised rats beat at a slower rate than atria from sedentary rats and that the atria from the trained rats were less sensitive to acetylcholine. Herrlich et al.[5] and de Schryver and Mertens-Strythagen[6] have reported increased amounts of acetylcholine in atria from exercised rats. Ekstrom[7] found increased activity of choline acetyltransferase in the atria of exercised rats indicating increased synthesis of acetylcholine. Tipton et al.[8] found no change in cholinesterase activity, but did find that trained rats had lower heart rates following an injection of neostigmine. To further investigate a possible cholinergic contribution to exercise bradycardia we have studied the response of isolated atria to atropine.

Methods. Male Sprague-Dawley rats were randomly assigned to control (sedentary) or experimental (exercised) groups. Exercised rats were run in a 6 compartment motor driven activity wheel twice daily for 1 h, 6 days per week.

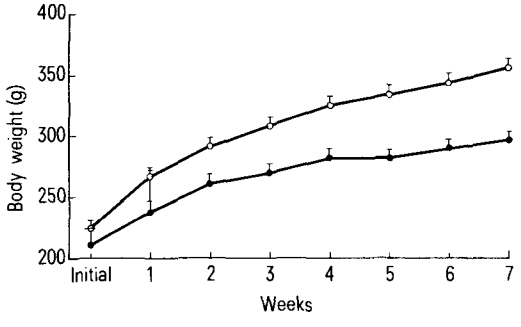

Fig. 1. Average b.wt of 12 sedentary (open circles) and 35 exercised rats (closed circles) over the 7-week exercise period. Extension bars represent the SEM.

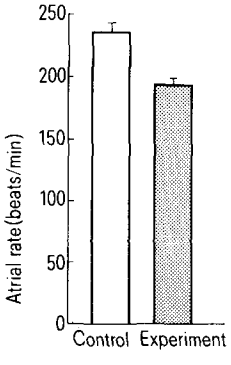

Fig. 2. Average rate of beating of isolated atria. Control, average rate of atria from 12 sedentary rats. Experiment, average rate of atria from 33 rats after 7 weeks of exercise. Extension bars represent SEM.

The training period lasted for 7 weeks. The rate of running was 12 m/min. Control rats were quartered with experimentals and were regularly placed in the activity cage, but were not exercised. All animals were weighed weekly. The animals were sacrificed at the end of the training period and the hearts rapidly removed into a dish of Krebs-Hensleit solution. The atria were dissected free and placed in a bath of Krebs-Hensleit solution (pH 7.2 ± 0.1) at 34 °C which was ocygenated continuously with 95% O_2, 5% CO_2. Atrial beats were recorded by means of a strain gauge with tension adjusted such that the atria were beating at the peak of the length-tension curve. The preparation was allowed to stabilize for a period of 0.5–1 h at which time the atrial rate was counted. The chronotropic response of atria to atropine was tested by adding the drug to the bath to bring the bath content of atropine to the following concentrations: 10^{-5} M, 10^{-6} M, 10^{-7} M. The atrial rate was then counted after stabilization. Statistical analyses were done according to methods described by Schefler[9].

Results. Body weight changes over the 7-week-period are shown in figure 1. We found no significant correlation between b.wt and the atrial rate. The spontaneous rate of atria isolated from sedentary rats was 235 ± 8.8 beats/min (mean ± SEM). The rate from exercised animals was 192 ± 5.8 beats/min (figure 2). The difference between these responses was significant ($p < 0.001$).

Figure 3 shows the responses of atria to atropine expressed as percent increase in rate. The slight increase in rate of control atria was not significant. In order to test whether the increase in rate of atria from exercised animals was significantly greater than that from sedentary animals we employed a factorial analysis of variance with atrial rate expressed as beats/min. This analysis was necessary since the basal rate of exercised atria was considerably lower than the basal rate of control atria. By this test, the response to atropine (with the exercise effect factored out) was significant when atropine was in a concentration of 10^{-5} M ($p < 0.01$) and in a concentration of 10^{-6} M ($p < 0.05$) but not in a concentration of 10^{-7} M. Interaction effects (between exercise and atropine factors) were, however, significant in all concentrations ($p < 0.001$) indicating that exercise has greatly heightened the chronotropic response to atropine. The rate of exercised atria in the presence of

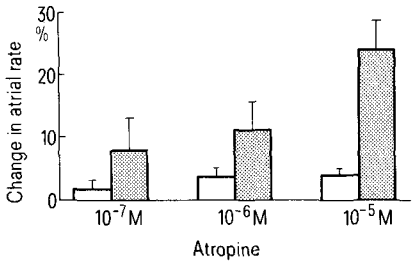

Fig. 3. Percent increase in rate of beating of isolated atria in the presence of the indicated concentrations of atropine. White bars, sedentary controls; black bars, exercised rats. In each case the bars represent averages from 6 sedentary controls and 16 exercised rats. Extension bars represent SEM.

10^{-5} M atropine (244 ± 12.8 beats/min) is not significantly different ($p > 0.5$) than the average basal rate (no atropine) of atria from sedentary animals (235 ± 8.8 beats/min).

Discussion. Exercise bradycardia cannot be attributed to an increase in vagal tone nor to a decrease in sympathetic tone since the decreased rate is present in atria isolated from exercised rats. The increased concentrations of acetylcholine in the heart reported by other workers[5,6], however, conceivably hold the rate of discharge of the pacemaker in abeyance, thus maintaining the bradycardia.

Our finding that atropine has no chronotropic effect on atria from sedentary animals is in agreement with the report of Grodner et al[10]. However, our finding of a significant increase in rate of atria from exercised animals in response to atropine has not been previously reported. The increases in atrial rate in response to atropine shift the atrial rate of exercised animals toward the basal atrial rates of sedentary animals, with 10^{-5} M atropine causing a rate in exercised atria which is not significantly different from the basal rate of atria from sedentary rats. These results may indicate that the increased amount of acetylcholine in atrial tissue reported by other workers plays an important role in producing exercise bradycardia.

1 This study was supported by a grant from the Genesee Valley Heart Association.
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Concentration by the renal sac of *Molgula manhattensis* of homarine, a nitrogenous compound

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Summary. An important compound in the renal sac fluid of *Molgula manhattensis* is homarine. This substance may have a role in the osmotic adjustment of the molgulid renal sac.

The renal sac of ascidians contains large amounts of uric acid and purine compounds[1-4]. It was considered for a long time as the storage-excretion structure of these animals[4-6]. The importance of this excretion was reconsidered after the work of Goodbody[7,8] who demonstrated that ascidians liberate ammonia in water. Goodbody showed that ammonia is the main product of nitrogenous excretion in ascidians as in other marine invertebrates. This excretion coexists with storage excretion, as Sabbadin et al.[9] confirmed. Other roles than simple excretion were predicted by Nolfi[10]. Nolfi showed the purine origin of uric acid in *Molgula manhattensis*, and suggested that it had a reserve function. A more recent work[11] concerns a possible osmoregulatory participation of the renal sac and shows that the ionic composition of renal sac fluid differs from that of blood. Organic compounds could be basically involved in osmotic adjustment. Determination of very large concentration of homarine in the renal sac fluid confirms the basic importance of this compartment. Homarine is a methyled base (figure 1), which contains little nitrogen and its role in nitrogenous excretion appears to be very weak in comparison with that of uric acid (figure 1). Homarine should, however, play a part in intracellular osmotic equilibrium as do other nitrogenous compounds[13-15].

Molgula manhattensis specimens were collected in the Bay of Arcachon. Adult animals were collected at different seasons (February, May, July). The renal sac is a closed system which contains fluid and concretions. Renal sac fluid and blood withdrawals can be made on the living animals with the organs in situ. Supernatant fluid and precipitate of both renal sac fluid and blood were analyzed after centrifugation. Analyses were also made on isolated renal concretions, renal envelope, intact renal sac and on the whole animal. The renal concretions were ground in cold 0.1 N perchloric acid. Homarine was identified by UV spectrophotometry (max = 272–273 nm) independent of the pH) and TLC on cellulose plates (Schleicher and Schull, 114 LS 254). Comigration with a reference substance (Homarine hydrochloride, Aldrich-Europe, No. 16037-7) was checked in several solvent systems (e.g. Propanol-ammonium hydroxide 1%, 2:1 v/v). Uric acid was identified by UV spectrophotometry (λ max = 286 nm at pH 1, 297 nm at pH 13), and determinated as previously described by one of us[16].

The major compound in the renal sac fluid is homarine (R_f 0.53). We detect 3 other UV absorbing more polar substances, all differing from uric acid (R_f 0.05). Homarine is present in very large amounts and it was necessary to dilute 500 times renal sac fluid for recording its UV-spectrum (figure 2). This substance is found at a level of 5.7 mg/ml of renal sac fluid. This amount corresponds to 3 mg/g of renal sac. Renal concretions show some traces of homarine and its concentration in the blood is about 0.2 mg/ml. Uric acid cannot be directly detected in the renal sac fluid by UV spectrophotometry. Its presence is noted, in very low amounts, after purification by TLC. We found on the average 2 mg uric acid for each renal sac, which represents 40% of concretions wet weight. Its presence in the renal

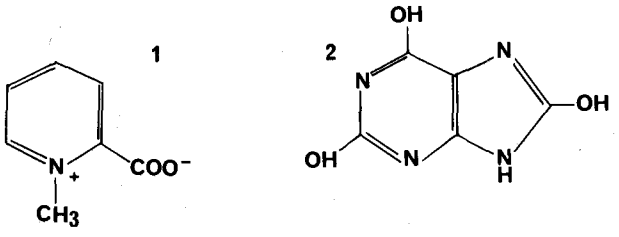

Fig. 1. Structural formula of homarine **1**, and uric acid **2**.