The Journal of Experimental Biology **199**, 1563–1568 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0343

AUTONOMIC NERVOUS CONTROL OF HEART RATE IN MUSKRATS DURING EXERCISE IN AIR AND UNDER WATER

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Accepted 21 March 1996

Summary

Neural control of the cardiac responses to exercise in air (running) and under water (diving) was studied in the muskrat (*Ondatra zibethicus*) by means of acute pharmacological blockade with the muscarinic blocker atropine and the β-adrenergic blocker nadolol. Saline injection was used as a control. Controls running on a treadmill showed a marked increase in heart rate with exercise. Atropine-treated animals had a higher resting heart rate than controls, but heart rate still increased with running. Nadolol-treated animals had a lower resting heart rate than controls and displayed a less pronounced increase in heart rate with running than controls. Animals treated with a combination of atropine and nadolol had a resting heart rate similar to that of controls but their heart rate was unaffected by running. Thus, exercise tachycardia in muskrats is due to activation of the sympathetic system and also to a reduction in parasympathetic tone. Heart rate decreased markedly during voluntary submergence in controls but rose as muskrats swam submerged against increasing water flows. Nevertheless, diving bradycardia was still present. Free-diving bradycardia and the relative increase in heart rate with underwater exercise were abolished by atropine and unaffected by nadolol. Hence, unlike the cardiac response to exercise in air, the cardiac response to underwater exercise is due only to a reduction in parasympathetic tone. Injection of the β-adrenergic agonist isoproterenol markedly increased heart rate in air but had little effect during voluntary and forced dives, indicating a marked decrease in the sensitivity of cardiac cells to adrenergic stimulation during submergence. These results strongly suggest that accentuated antagonism between the two branches of the autonomic nervous system occurs during diving so that parasympathetic influences on the heart predominate and inhibit any chronotropic response to adrenergic stimulation.

Key words: diving bradycardia, exercise tachycardia, muskrat, *Ondatra zibethicus*, telemetry, atropine, nadolol, isoproterenol, accentuated antagonism, sympathetic system, parasympathetic system.

Introduction

Diving mammals and birds exhibit a marked decrease in heart rate (f_H) during diving. The autonomic nervous control of free and forced diving bradycardia is now well documented (Butler and Jones, 1971; Furilla and Jones, 1987; Murdaugh *et al.* 1961; Signore and Jones, 1995). Diving bradycardia is caused by an increase in parasympathetic activity and sympathetic influences have little effect. Equally well known is the nervous control of the cardiac response of terrestrial mammals to exercise in air (Ekblom *et al.* 1972; Robinson *et al.* 1966; Stramba-Badiale *et al.* 1991; Yamamoto *et al.* 1991). Exercise tachycardia in air is mainly due to an increase in sympathetic activity and, to a lesser extent, to a decrease in parasympathetic activity. However, nothing is known about the autonomic nervous control of f_H when diving and exercise responses are in play at the same time. This issue is of particular importance because it has been proposed that the intensity of diving bradycardia during voluntary dives is related to the degree of exercise performed by the experimental subject and that free-diving bradycardia is, in fact, a compromise response resulting from the opposing effects of exercise and forced diving responses (Millard *et al.* 1973; Woakes and Butler, 1983).

In the present study, the autonomic nervous control of f_H during exercise in air and under water was investigated in muskrats (*Ondatra zibethicus*). f_H of muskrats running on a treadmill and swimming voluntarily under water against a variable water flow was monitored using telemetry. The nervous control of the cardiac response in both types of exercise was then compared using pharmacological blockade of muscarinic and β-adrenergic receptors. Muskrats were acutely treated with the muscarinic blocker atropine and the β-adrenergic blocker nadolol and the effects of the injected drugs on f_H were studied during exercise in air and under water. Finally, the sensitivity of cardiac cells to adrenergic stimulation in air and under water was investigated by treating muskrats with the β-adrenergic agonist isoproterenol.

Materials and methods

Eleven adult muskrats (nine males and two females), ranging in mass from 0.7 to 1.2 kg, were used in the present experiments. Muskrats *Ondatra zibethicus* (L.) were trapped in Surrey, British Columbia, and housed in pairs in 76 cm×51 cm×41 cm cages at the Animal Care Centre of the University of British Columbia. They were fed with laboratory rodent diet (LabDiet 5001, PMI Feeds, St Louis, MI, USA) supplemented with carrots; each pair had access to a 28 cm×18 cm×13 cm tank filled with running water.

Muskrats were anaesthetized with a mixture of 2 mg kg^{-1} acepromazine (AC Promazine, Austin Laboratories, Joliette, Quebec) and 40 mg kg^{-1} ketamine (Ketalean, M.T.C. Pharmaceuticals, Cambridge, Ontario) injected subcutaneously. The eyes were protected with ophthalmic ointment (Neosporin, Burroughs Wellcome, Kirkland, Quebec) during anaesthesia. Fur was clipped from the area where incisions were to be made and the exposed skin was cleaned with Betadine (Purdue Frederick, Pickering, Ontario). Electrocardiogram (ECG) transmitters (Konigsberg Instruments, Pasadena, CA, USA) were implanted in the peritoneal cavity. Transmitter leads were threaded through the peritoneal wall and then subcutaneously over the thoracic cavity, where they were sutured to the ribs. Following surgery, antibiotic powder (Cicatrin, Burroughs Wellcome, Kirkland, Quebec) was applied to skin incisions and 50 mg kg^{-1} oxytretracycline (Liquamycin LA, Rogar/STB, Montreal, Quebec) was injected subcutaneously. Experiments were not started until at least 1 week after surgery. All procedures were approved by the Animal Care Committee of the University of British Columbia.

The following treatments were used: injection of saline (control), injection of the muscarinic antagonist atropine sulphate (0.5 mg kg^{-1}, Sigma, St Louis, MI, USA), the β-adrenergic antagonist nadolol (8 mg kg^{-1}, Sigma) and the β-adrenergic agonist isoproterenol hydrochloride (0.05 mg kg^{-1}, 0.5 mg kg^{-1} or 5 mg kg^{-1}, Sigma). To check for a possible effect of arousal on f_H due to the increased level of noise with water flow in the flume, a treatment group of muskrats tranquilized with the benzodiazepine diazepam (1 mg kg^{-1}, Diazemuls, KabiVitrum, Newmarket, Ontario) was also used. Drugs were mixed in 0.9 % saline and injected subcutaneously. This route of injection was chosen because it gave a reliable blockade as shown by the absence of cardiac effects of agonists in treated animals (see below), it required little handling of the animals and, unlike intramuscular injections, it did not seem to impair locomotion. The doses of atropine, nadolol, isoproterenol (0.05 mg kg^{-1}) and diazepam used in the present study were selected because they were found to be the largest doses under which muskrats dived voluntarily. Isoproterenol doses of 0.5 mg kg^{-1} and 5 mg kg^{-1} could be used only in animals forced to dive. Tests using the saline, nadolol and diazepam groups lasted 2 h whereas tests with the atropine group lasted 1 h and with the isoproterenol group lasted 30 min, because of the shorter half-lives these two drugs. Different treatments were administered at least 2 days apart. To check for efficacy of blockade, f_H was monitored after subcutaneous injection of the cholinergic agonist pilocarpine hydrochloride (1 mg kg^{-1}, Sigma) at the end of exercise after atropine treatment and of the β-adrenergic agonist isoproterenol hydrochloride (0.05 mg kg^{-1}, Sigma) after nadolol treatment. The efficacy of double blockade with atropine and nadolol was checked by monitoring f_H after subcutaneous injection of pilocarpine hydrochloride (1 mg kg^{-1}) followed, 15 min later, by injection of isoproterenol hydrochloride (0.05 mg kg^{-1}). The effects of agonists injected alone, before blockade, were assessed in three animals. Pilocarpine decreased resting f_H by 20 % and isoproterenol increased resting f_H by 60 %. In all cases, the effects of agonists on f_H were blocked in antagonist-treated muskrats.

Cardiac response to exercise during free diving was investigated in five muskrats. Animals could rest in a 52 cm× 34 cm×26 cm plastic mesh cage located above a variable-speed water flume that they could enter at one end from a ramp. Muskrats dived voluntarily against the water flow to get to food placed under water at the other end of the water flume (2.5 m round trip). The 21 cm wide water channel was filled to a depth of 20 cm with water. Water velocity, determined by timing a neutrally buoyant particle across a known distance, was set to 0, 0.3, 0.6 or 0.9 m s^{-1} in randomized order. Water temperature ranged from 8 to 12 °C. Data collection started as soon as muskrats were trained to dive at all water velocities. Training took about 3 days. Before each exercise test, animals were placed in the plastic mesh cage positioned above the water flume and were not given access to the water for 15 min to allow them to adjust to their situation. Drugs or saline were then injected and access to the water flume was allowed. Water velocity was controlled remotely and no observer was present during free-diving sessions. All five animals received the following treatments in randomized order: injection of saline, atropine, nadolol or diazepam. In addition, to study the cardiac responses to adrenergic stimulation in air and under water, the effect of isoproterenol (0.05 mg kg^{-1}) on f_H in air and during voluntary dives in still water was studied in the flume.

Cardiac response to running was investigated in six muskrats. Animals were placed on a treadmill and had to run for 30 s at speeds of 0.4, 0.8 and 1.2 m s^{-1}. The highest speed of 1.2 m s^{-1} was chosen as it was the maximum speed at which the slowest muskrat could run after 2 weeks of training. The other two speeds were then chosen as one-third and two-thirds of this maximum speed. The surface of the treadmill on which muskrats ran was 83 cm×33 cm and was covered by a 22 cm high plastic mesh cage. The treadmill was operated remotely and no observer was present in the room during running sessions. Data collection started as soon as animals were trained to run for 30 s at each speed, which took 1–2 weeks. Before each exercise test, animals were allowed to warm up by running for 30 s at each speed. Saline or drugs were then injected and the first run of the test was performed 5 min later. Each running session consisted of four 30 s runs at each speed performed in randomized order. A stable f_H was reached within the first 15 s of each run. Muskrats were allowed 3–4 min to

rest between runs. All six animals received the following treatments in randomized order: injection of saline, atropine, nadolol, diazepam and a combination of atropine and nadolol.

To provide better control of the time of submergence after injection of isoproterenol and to study the effects of higher doses of isoproterenol, f_H was monitored in six muskrats during forced dives performed 5–10 min after injection of 0.05, 0.5 and 5 mg kg^{-1} isoproterenol. Saline was injected as a control. Forced dives were performed in a 58 cm×36 cm×23 cm plastic mesh cage submerged in a 91 cm×46 cm×43 cm aquarium. Water temperature ranged from 8 to 12 °C. Muskrats were left for 15 min in air in the cage before the beginning of the session. The drug was administered and one dive was performed 5–10 min later. Dive length ranged from 52 to 64 s. The experimenter entered the room about 30 s before a dive and left the room within the first 15 s of recovery from the dive.

The ECG was monitored using a Konigsberg Instrument telemetry system (Pasadena, CA, USA). The ECG signal was recorded on the audio channel of a video cassette recorder after modulation using a Vetter FM recording adaptor (A. R. Vetter Co., Rebersburg, PA, USA). The behaviour of the animal was recorded on the video channel using a Panasonic camera (Secausus, NJ, USA). When tapes were replayed, the ECG signal was demodulated and logged using Labtech Notebook software (Laboratory Technologies Corporation, Wilmington, MA, USA) running on an IBM-compatible personal computer that calculated and stored inter-beat intervals. An event marker was connected to the computer to record the time at which the animal dived and surfaced, or started and stopped running, judged from watching the animal on a television monitor. Subsequently, each inter-beat interval was converted to beats min^{-1}.

Mean heart rate over 30 s was calculated after treatment during periods of rest (resting f_H) in the water flume, in the forced diving cage or on the treadmill. Mean values from each muskrat were averaged to give a grand mean for resting f_H (N=5 in the water flume, N=6 in the forced diving cage and on the treadmill). In the water flume, mean heart rate during free diving (diving f_H) as well as mean heart rate during the 15 s preceding a dive (pre-dive f_H) and following a dive (post-dive f_H) were calculated for each dive. To reduce variability caused by dive duration, only the first four voluntary dives lasting more than 15 s and less than 30 s were analysed for each animal at each water velocity. Values from the four dives at each water velocity were averaged in each animal to give a mean for pre-dive f_H, dive f_H and post-dive f_H. Mean values from each animal (N=5) were then averaged in each group to give a grand mean for each variable at each water velocity. Mean heart rate during running was calculated for each of the four runs at each speed. Values from the four runs at each speed were averaged in each animal to give a mean for f_H during running at each speed. Mean values from each animal (N=6) were averaged to give a grand mean for f_H during running at each speed. Resting f_H, pre-dive f_H, diving f_H and post-dive f_H were calculated for each forced dive. Averages for 5 s periods, starting 20 s before forced dives and ending 20 s after forced dives, were also computed to study heart rate profiles during forced dives. Mean values from each muskrat (N=6) were averaged to give a grand mean for each variable.

Values given in the text are grand mean ± S.E.M. One-way and two-way analyses of variance (ANOVAs) for repeated measures were computed, and multiple comparisons were performed using Student–Newman–Keuls tests. Overall effects and differences were considered significant when P<0.05. All statistics were carried out using SigmaStat software (Jandel Scientific, San Rafael, CA, USA).

Results

Effect of water velocity on f_H *in saline-injected muskrats*

Saline-injected animals had a resting f_H of 234±11 beats min^{-1} in the cage above the water flume. Pre-dive f_H and post-dive f_H were significantly higher than resting f_H and diving f_H was significantly lower (Fig. 1). Pre-dive f_H and post-dive f_H were unaffected by increases in water velocity while diving f_H increased significantly with water velocity (Fig. 1). Diving f_H at a water velocity of 0.9 m s^{-1} (169±25 beats min^{-1}) remained significantly lower than resting f_H.

Effect of drugs on f_H *in the water flume (still water)*

Atropine significantly increased resting f_H to 296±6 beats min^{-1}. Resting f_H in nadolol-treated (196±13 beats min^{-1}) and diazepam-treated muskrats (224±7 beats min^{-1}) was not significantly different from resting f_H in saline-injected animals. Injection of atropine significantly increased post-dive f_H and diving f_H but not pre-dive f_H (Fig. 1). Diving f_H in atropine-treated animals was not significantly different from their pre-dive f_H and post-dive f_H. Injection of nadolol significantly decreased pre-dive f_H and post-dive f_H but had no significant effect on diving f_H (Fig. 1). Injection of diazepam had no significant effect on pre-dive f_H, post-dive f_H and diving f_H (Fig. 1).

Effects of drugs on f_H *during underwater exercise*

As in saline-injected muskrats, pre-dive f_H and post-dive f_H in drug-treated animals were unaffected by increases in water velocity (Fig. 1). Diving f_H at any given water velocity was unaffected by treatment with diazepam and nadolol, showing the same significant increase with water velocity as in saline-injected muskrats (Fig. 1). Atropine-treated muskrats did not show any significant increase in diving f_H with increasing water velocity (Fig. 1).

Effect of drugs on f_H *during running*

Resting f_H on the treadmill was 209±8 beats min^{-1} in saline-injected animals and f_H rose significantly with running speed (Fig. 2). Atropine significantly increased resting f_H to 266±5 beats min^{-1}. f_H during running increased significantly over resting f_H in atropine-treated muskrats (Fig. 2). Nadolol significantly decreased resting f_H to 175±8 beats min^{-1}. f_H during running increased significantly over resting f_H in nadolol-treated muskrats (Fig. 2). Resting f_H in muskrats

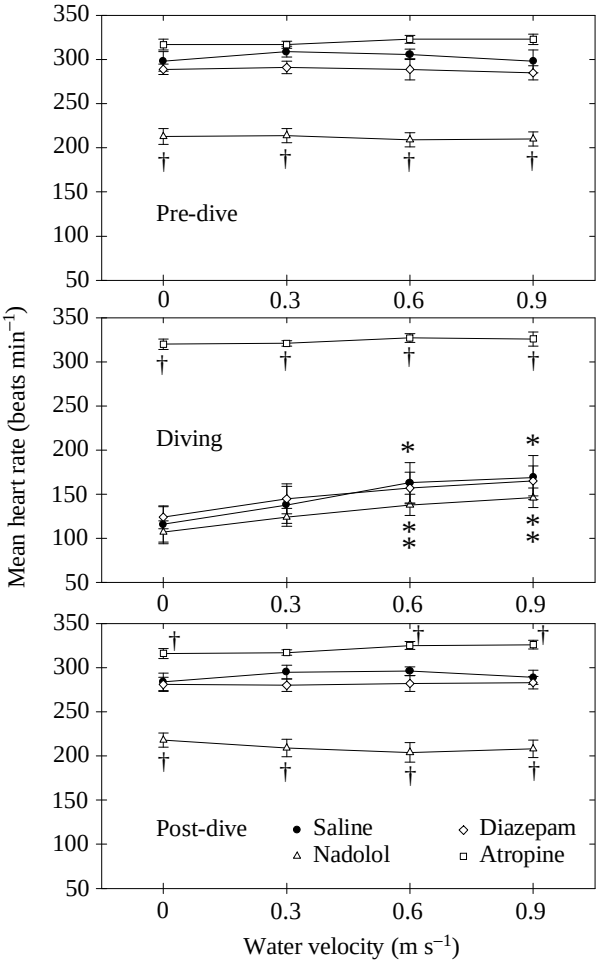

Fig. 1. Effect of water velocity and injected drugs on mean heart rate f_H (±S.E.M., N=5) during voluntary submergence. Pre-dive f_H, diving f_H and post-dive f_H are shown for muskrats treated with saline (control), atropine, nadolol and diazepam. * indicates values significantly different from f_H in still water in the same treatment group. † indicates values significantly different from f_H in saline-injected animals at the same water velocity.

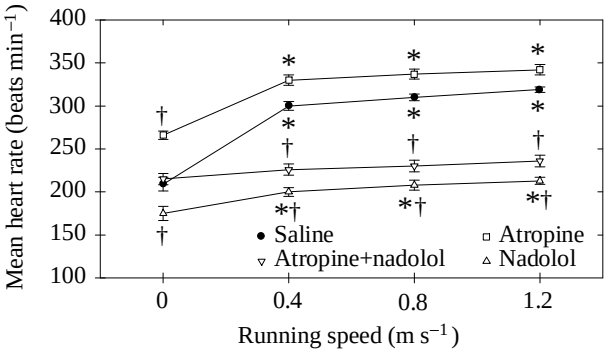

Fig. 2. Effect of running speed on mean heart rate f_H (±S.E.M., N=6) in muskrats treated with saline (control), atropine, nadolol and a combination of atropine and nadolol. * indicates values significantly different from resting f_H in the same treatment group. † indicates values significantly different from f_H in saline-injected animals running at the same speed.

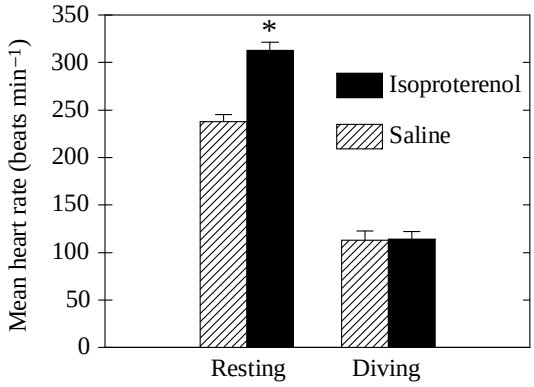

Fig. 3. Effect of isoproterenol (0.05 mg kg^{-1}) on mean heart rate f_H (+S.E.M., N=5) in muskrats resting in air and diving voluntarily. * indicates values significantly different from f_H in saline-injected animals in the same situation.

treated with a combination of nadolol and atropine (215±7 beats min^{-1}) was not significantly different from resting f_H in saline-injected animals and their f_H was unaffected by running (Fig. 2). Four out of the six diazepam-treated muskrats were not able to run, even at the slowest speed. The other two animals showed a significant increase in f_H during running compared with their resting f_H (184±12 beats min^{-1}): their f_H was 248±6 beats min^{-1} during running at a speed of 0.4 m s^{-1}, 257±10 beats min^{-1} at 0.8 m s^{-1} and 272±5 beats min^{-1} at 1.2 m s^{-1}.

Effect of isoproterenol on f_H *in the water flume*

Resting f_H was 238±7 beats min^{-1} in saline-injected animals and it increased significantly in isoproterenol-treated muskrats (Fig. 3). Isoproterenol increased pre-dive f_H (310±5 beats min^{-1}) and post-dive f_H (306±6 beats min^{-1}) compared with pre-dive f_H (281±13 beats min^{-1}) and post-dive f_H (274±8 beats min^{-1}) in saline-injected animals, but the increase was significant only for post-dive f_H. Injection of isoproterenol had no significant effect on free-diving f_H (Fig. 3).

Effect of isoproterenol on f_H *in the forced diving cage*

None of the three concentrations of isoproterenol had any significant effect on heart rate profiles during forced dives. f_H averaged 238±13 beats min^{-1} in the 5 s preceding the dive in saline-injected animals. It dropped markedly on submergence to reach 27±3 beats min^{-1} at 10 s into the dive, stayed low until the end of the dive and came back to 233±12 beats min^{-1} within the first 5 s after the dive. Fig. 4 illustrates the effect of the three concentrations of isoproterenol on f_H in air and during forced dives. Injection of 0.05 mg kg^{-1} isoproterenol significantly increased resting f_H and increasing the concentration of isoproterenol did not have any further effect on resting f_H. In contrast, injection of isoproterenol did not induce any significant change in forced diving f_H at any concentration.

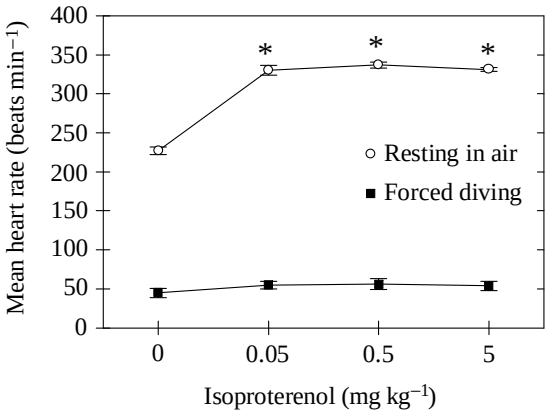

Fig. 4. Effect of three concentrations of isoproterenol on mean heart rate f_H (±S.E.M., N=6) in muskrats resting in air and forcibly submerged. * indicates values significantly different from f_H in saline-injected animals (0 mg kg^{-1} isoproterenol) in the same situation.

Discussion

Muskrats diving voluntarily in still water displayed a marked bradycardia (Drummond and Jones, 1979; MacArthur and Karpan, 1989; McCulloch and Jones, 1990; Signore and Jones, 1995). Diving f_H increased when muskrats had to swim against increasing water velocities but a diving bradycardia was still present at a water velocity of 0.9 m s^{-1}, which corresponds to a swimming speed approximately 50 % above the surface swimming speed chosen voluntarily by muskrats (0.58 m s^{-1}; Fish, 1982). Muskrats swimming under water at 0.9 m s^{-1} appear to be close to their maximum speed so it is unlikely that underwater exercise could ever be intense enough to drive diving f_H to resting values.

Previous studies in diving ducks and penguins suggested that the level of activity might explain differences in f_H seen during different types of dive (Millard *et al.* 1973; Woakes and Butler, 1983). In muskrats, voluntary dives are accompanied by a bradycardia that is intensified during escape dives and even more pronounced during forced dives (McCulloch and Jones, 1990). Diving f_H is also lower in exploratory dives than in more active foraging dives (MacArthur and Karpan, 1989). The results of the present experiments show that exercise can indeed increase free-diving f_H to some extent in muskrats. However, forcibly submerged muskrats can exhibit a much more profound diving bradycadia than freely diving muskrats despite swimming around in the forced diving cage (Signore and Jones, 1995). Hence, other factors such as the presence of an experimenter or the absence of control of dive duration probably affect f_H during forced dives in muskrats.

The increased level of noise with water flow in the water flume could have been responsible for some of the change in diving f_H with increasing water velocity since our previous research suggested that the level of arousal might affect free-diving bradycardia (Signore and Jones, 1995). Muskrats tranquilized with diazepam displayed the same increase in diving f_H with water velocity as controls, confirming that diving f_H increased as a result of exercise and not arousal. We are confident that the amount of diazepam used had a central effect because all diazepam-treated muskrats were easy to handle and frequently lost their balance in air (they were surprisingly at ease under water), and also because four out of six diazepam-treated muskrats could not run on the treadmill even at the lowest speed.

Blockade of the β-adrenergic system with nadolol had no effect on the increase in diving f_H during underwater exercise. This is a surprising finding because exercise tachycardia in air has been shown, in man and dogs, to be mainly due to activation of the sympathetic system and only to a lesser extent to withdrawal of parasympathetic tone (Ekblom *et al.* 1972; Robinson *et al.* 1966; Stramba-Badiale *et al.* 1991). One possible explanation is that the nervous control of the cardiac response to exercise is different in muskrats from that in man and dogs. Our results in running muskrats show that this is not the case. Atropine had little effect on running tachycardia whereas nadolol markedly reduced it and a combination of atropine and nadolol abolished it. These results emphasize the role of sympathetic stimulation during running and are similar to those obtained in man and dog. The absence of an effect of sympathetic blockade during voluntary diving despite an effect during the pre-dive and post-dive periods has previously been observed in muskrats and led us to hypothesize that the parasympathetic system overrides any sympathetic influence on the heart (accentuated antagonism) during diving (Signore and Jones, 1995). Therefore, it is difficult to assess what happens to sympathetic outflow to the heart during underwater exercise since its cardiac chronotropic effect is blocked by the parasympathetic system. However, results from atropine-treated muskrats shed some light on this question. These animals exhibited a high diving f_H similar to their pre-dive f_H and post-dive f_H, confirming that free-diving bradycardia is indeed caused by the parasympathetic system and that sympathetic activity, which is high during the pre-dive and post-dive periods, does not collapse during diving (Signore and Jones, 1995). Furthermore, diving f_H in atropine-treated muskrats was unaffected by underwater exercise, suggesting that, even if sympathetic outflow to the heart increases during underwater exercise, diving f_H cannot be raised in atropine-treated muskrats because f_H is close to its maximum value. In fact, diving f_H in atropine-treated muskrats is similar to f_H in muskrats running at high speed and to f_H in resting muskrats treated with massive doses of the β-agonist isoproterenol. This tends to confirm our suggestion that diving f_H in atropine-treated muskrats is maximal.

Accentuated antagonism can be the result of parasympathetic inhibition of the release of catecholamines by cardiac sympathetic nerve terminals and/or parasympathetically mediated insensitivity of cardiac cells to catecholamines (Carrier and Bishop, 1972; Kimura *et al.* 1985; Levy, 1971). Results from isoproterenol-treated muskrats suggest that the second mechanism occurs during diving. Isoproterenol markedly increased f_H in resting animals. This was probably due to stimulation of cardiac β-receptors and also to reflex tachycardia resulting from stimulation of vascular β-adrenergic receptors causing vasodilation and a fall in blood pressure (Katzung, 1992). Cardiac effects of isoproterenol were not

apparent during voluntary and forced dives, even at high concentrations, indicating a marked decrease in sensitivity of cardiac cells to adrenergic stimulation during diving. It is interesting to note that ducks and seals forced to dive also exhibit a marked diving bradycardia despite a remarkable increase in circulating catecholamines during submergence (Hance *et al.* 1982; Lacombe and Jones, 1990). The same parasympathetic inhibition of the cardiac response to catecholamines may be present in those species as well.

Accentuated antagonism between the parasympathetic and sympathetic systems has been extensively investigated only in isolated heart preparations and anaesthetized animals (Carrier and Bishop, 1972; Grodner *et al.* 1970; Levy and Zieske, 1969; Levy *et al.* 1966). One study made use of an awake animal model but vagal stimulation was artificially induced (Stramba-Badiale *et al.* 1991). The present experiments suggest that a cardiac accentuated antagonism between the two branches of the autonomic nervous system may occur naturally in an awake animal.

In conclusion, these experiments show that the level of exercise can increase free-diving f_H in muskrats but that a diving bradycardia is still present. They also demonstrate that the increase in f_H with underwater exercise is due to withdrawal of parasympathetic tone whereas exercise tachycardia in air is mainly caused by activation of the sympathetic system and, only to a lesser extent, by reduction of parasympathetic outflow to the heart. Finally, results in isoproterenol-treated muskrats strongly suggest that the nervous control of the cardiac response to exercise under water is different from that in air because of an accentuated antagonism between the two branches of the autonomic nervous system during diving so that parasympathetic influences on the heart inhibit the cardiac chronotropic response to adrenergic stimulation.

This research was supported by research grants from the Natural Sciences and Engineering Research Council of Canada and the Heart and Stroke Foundation of British Columbia and Yukon. The authors wish to thank Dr A. T. Mosberg from R. J. Reynolds Tobacco Co. (Winston-Salem, NC, USA) for lending us the ECG telemetry system and Dr J. M. Gosline from the University of British Columbia (Vancouver, BC, Canada) for lending us the water flume used in these experiments.

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