# The Autonomic Nervous Control of Heart Rate in Ducks during Voluntary Diving

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Accepted 8/17/98

## ABSTRACT

Autonomic nervous control of heart rate was studied in voluntarily diving ducks (*Aythya affinis*). Ducks were injected with the muscarinic blocker atropine, the $\beta$-adrenergic blocker nadolol, the $\beta$-adrenergic agonist isoproterenol, and a combination of both atropine and nadolol. Saline injection was used as a control treatment. The reduction in heart rate (from the predive level) normally seen during a dive was abolished by atropine. Nadolol reduced heart rate during all phases of diving activity—predive, dive, and postdive—indicating that sympathetic output to the heart was not withdrawn during diving. Isoproterenol increased heart rate before, during, and after the dive, although the proportional increase in heart rate was not as high during the dive as compared with the increase in routine heart rate or heart rate during the predive or postdive phase. The parasympathetic system predominates in the control of heart rate during diving despite the maintenance of efferent sympathetic influences to the heart, perhaps due to accentuated antagonism between the two branches of the autonomic nervous system.

## Introduction

The control of heart rate ($f$H) by the autonomic nervous system during forced and voluntary diving has been studied extensively (Murdaugh et al. 1961; Butler and Jones 1971; Furilla and Jones 1987; Signore and Jones 1995). Cardiac adjustments during voluntary diving are dominated by parasympathetic vagal output in seals and muskrats (Murdaugh et al. 1961; MacArthur and Karpan 1989; Signore and Jones 1995). In ducks, vagal output from the parasympathetic nervous system is responsible for the reduction in $f$H during forced and escape dives (Butler and Woakes 1982), although the precise role of the parasympathetic system in the control of $f$H in ducks during voluntary dives has yet to be substantiated. $\beta$-adrenergic blockade using propranolol in voluntarily diving ducks demonstrated the importance of the sympathetic system in the elevation of $f$H in the predive phase of a dive cycle, but no effect was seen on $f$H during the dive (Furilla and Jones 1987).

In the present study, we investigated the autonomic nervous control of $f$H during voluntary diving in ducks (*Aythya affinis*). Routine $f$H and $f$H before, during, and after the dive were monitored using telemetry. The effect on $f$H of the elimination of sympathetic influences using the $\beta$-adrenergic blocker nadolol and the removal of parasympathetic input to the heart with the muscarinic blocker atropine was tested. In addition, the effects on $f$H of using a combination of both atropine and nadolol were investigated. Finally, the effect on $f$H during voluntary diving was studied after stimulation of the sympathetic system with the $\beta$-adrenergic agonist isoproterenol.

## Material and Methods

Two female and three male adult diving ducks (*Aythya affinis*) with an average mass of 636 $\pm$ 18 kg (SEM) were used in these experiments. The ducks were raised and housed in the South Campus Animal Care Center (Zoology) at the University of British Columbia. Ducks were kept together in a pen 1.6 × 0.6 × 0.9 m, with free access to a tank of water 2.5 × 1.25 × 0.8 m. Most of the surface of the tank (1.3 × 0.8 m) was covered by wire mesh except the end nearest to the pen, which restricted the ducks to diving from and resurfacing at the same place. Wheat (Buckerfield's, Abbotsford, British Columbia) was put on the bottom of the tank to encourage diving. During the training period, the wheat was moved from directly under the open area of water surface to the opposite end of the tank. Goose and duck pellets (Buckerfield's) were provided in limited amounts in the pen as a dietary supplement.

Electrocardiogram (ECG) transmitters (Narco Proprietary Design, Downsview, Ontario) were embedded in an epoxy encapsulate (Sealtronic epoxy encapsulate, Industrial Formulators of Canada, Burnaby, British Columbia). The transmitters were equipped with a 3.5-volt button-tab battery (Biotelemetrics, Boca Raton, Fla.) and a miniature magnetic reed switch (Biotelemetrics). The battery and switch were then covered with a

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*Physiological and Biochemical Zoology* 72(2):164–169. 1999.  1522-2152/99/7202-9858$03.00

mixture of paraffin and beeswax, and the entire device coated with silicone (G.E. RTV 118, Winford Insulation, Burnaby, British Columbia). The transmitter leads consisted of 18-gauge hypodermic needles shaped into a loop and pressed onto the end of stainless-steel biomedical wire (Cooner Wire, Chatsworth, Calif.). Connections between the wire and the loop were sealed with silicone.

For surgical anesthetization, ducks were intubated and ventilated with 98% oxygen and 2% Halothane (Fluothane, Wyeth-Ayerst, Montreal). Exposed skin at the site of incision (the midline of the ventral abdominal wall, just below the caudal end of the sternum) was cleaned with Betadine (Purdue Fredrick, Pickering, Ontario). The transmitter was implanted in the peritoneal cavity and sutured to the abdominal wall. The two transmitter leads were pushed rostrally through a small hole made in the oblique septum and were placed one on each side of the heart. Following surgery, an antibiotic powder (Cicatrin, Burroughs Wellcome, Kirkland, Quebec) was applied to the incision, and 2 mL $kg^{-1}$ of Tribrissen antibiotic (Coopers Agropharm, Ajax, Ontario) were injected into the pectoral muscle.

Experiments were started 1–2 wk after surgery when the animals were diving normally. During diving sessions, ECG was monitored using an FM receiver (Model 555, Sony, Vancouver, Canada). The ECG signal was modulated using a FM recording adapter (A. R. Vetter, Rebersberg, Pa.) to enable the signal to be recorded on the audio channel of a video recorder. A camera (Panasonic, Secausus, N.J.) was used to record the duck's diving behavior on the video channel. The videotapes were replayed, the ECG signal was demodulated, and interbeat intervals calculated on an IBM-compatible computer using Labtech Notebook software (Laboratory Technologies, Wilmington, Mass.). The interbeat intervals were subsequently converted into $f$H (beats $min^{-1}$). An event marker was connected to the computer to allow correlation of diving activity on the video channel with heart rate on the audio channel during review of the videotape. Each dive cycle was divided into three stages: predive, dive, and postdive.

The study commenced with the control trials (saline injection) to ensure proper functioning of the transmitter and to reinforce the training. The ducks then received the following treatments in no particular order: injection of the muscarinic antagonist atropine sulphate (2 mg $kg^{-1}$, Sigma, St. Louis), the $\beta$-adrenergic antagonist nadolol (2 mg $kg^{-1}$, Sigma), the $\beta$-adrenergic agonist isoproterenol hydrochloride (1 mg $kg^{-1}$, Sigma), and a mixture of atropine (2 mg $kg^{-1}$) and nadolol (2 mg $kg^{-1}$). Drugs were administered in 0.9% saline and injected into the pectoral muscle. Ducks were immediately returned to the tank and left for 5 min to recover from handling. Diving sessions for control groups lasted 6 h, sessions for nadolol lasted 4 h, and sessions for atropine and the mixture of atropine and nadolol lasted for 1 h because of the short half-life of atropine. Different drug treatments were administered at least 2 d apart. Drug levels were tested for efficacy of blockade by injection of an agonist. Agonists were tested alone before blockade with their respective antagonists. The $\beta$-adrenergic agonist isoproterenol hydrochloride (1 mg $kg^{-1}$, Isuprel, Sterling-Winthrop, Markham, Ontario) injected into the pectoral muscle increased $f$H. The cholinergic agonist acetylcholine chloride (1 mg $kg^{-1}$, Sigma) injected into a wing vein caused a brief but massive reduction in $f$H. Treatment with the appropriate antagonists prevented any effects on $f$H of the corresponding agonist.

Throughout a voluntary dive, there is a progressive cardioinhibitory effect of the carotid body chemoreceptors on heart rate in tufted ducks (Butler and Woakes 1982). Therefore, only dives lasting more than 8 s and less than 21 s were analyzed in an effort to reduce variability caused by dive duration. Dive durations averaged 13–17 s in the treatment groups (see "Results"), which is similar to the average length of a natural voluntary dive (ca. 15 s) (Butler and Woakes 1982). Six dives meeting these temporal criteria from each duck for each condition (control, atropine, nadolol, and mixture) were chosen and analyzed. For the isoproterenol treatment, four dives were analyzed from three ducks and compared with saline injection (control) in the same three animals. Routine $f$H values represent the mean of three 30-s periods after each treatment, when the duck was quietly paddling on the water surface. Mean $f$H for each dive phase was then determined. Shortly before the predive phase, $f$H increases from routine $f$H levels; therefore, the mean $f$H for only 5 s immediately preceding the dive was used to give predive $f$H. For the dive phase, mean $f$H was averaged for the entire time of submergence. Postdive $f$H was obtained by analyzing only the first 5 s following surfacing because $f$H falls rapidly after the initial tachycardia seen on the ducks' return to the water surface. Averaging $f$H determined for each set of dives gave a mean for each treatment condition. Means for all ducks were then averaged to give a group mean. The difference in the average of five heartbeats near the start of a dive (the five beats following the initial three after submersion) and five heartbeats at the end of the dive (not including the last one before emersion) for each treatment was used as an indication of the change in $f$H throughout the dive. An additional measure of the effect of isoproterenol on $f$H was obtained by subtraction of $f$H in the control condition (saline treatment) from $f$H in the corresponding phase during the isoproterenol treatment.

For statistical analysis of the data, one-way ANOVA for repeated measures was computed. Multiple comparison procedures (Student-Newman-Kuels Method) were performed. All statistics were calculated with SigmaStat software (Jandel Scientific, San Rafael, Calif.) with $P < 0.05$ considered significantly different.

## Results

Dive times for the saline and the drug treatments are shown in Table 1. Since the dives were chosen to fit specific temporal

criteria (see "Material and Methods"), no significant differences in any of the dive times for the five treatments were expected.

The *f*H profiles of a duck diving under the four treatment conditions—saline, atropine, nadolol, and a mixture of atropine and nadolol—are shown in Figure 1. The effect of injected drugs on the group mean averages for *f*H in routine, predive, and postdive phases are shown in Figure 2. There was a significant effect on routine *f*H of all drug treatments compared with saline injection (controls; $f\text{H} = 190 \pm 18$ beats $\text{min}^{-1}$). Blockade of vagal influence to the heart with atropine increased routine *f*H ($373 \pm 20$ beats $\text{min}^{-1}$) and sympathetic blockade with nadolol lowered routine *f*H to $141 \pm 18$ beats $\text{min}^{-1}$ (Fig. 2). A mixture of both atropine and nadolol resulted in a routine *f*H of $322 \pm 20$ beats $\text{min}^{-1}$, considerably elevated above the control value (Fig. 2).

Predive *f*H ($326 \pm 10$ beats $\text{min}^{-1}$) in the controls increased to $377 \pm 21$ beats $\text{min}^{-1}$ after injection of atropine (Fig. 2). Following nadolol injection, predive *f*H ($263 \pm 10$ beats $\text{min}^{-1}$) fell significantly compared with the other three treatments (Fig. 2). Predive *f*H after injection of a mixture of both atropine and nadolol was $330 \pm 17$ beats $\text{min}^{-1}$ (Fig. 2).

Diving *f*H differed significantly in all drug treatments compared with ducks given saline injections (controls). Atropine increased diving *f*H to $378 \pm 22$ beats $\text{min}^{-1}$ compared with *f*H after saline injection ($200 \pm 18$ beats $\text{min}^{-1}$) (Fig. 2). Nadolol lowered dive *f*H ($155 \pm 15$ beats $\text{min}^{-1}$) and the mixture of both atropine and nadolol resulted in a diving *f*H of $321 \pm 19$ beats $\text{min}^{-1}$ (Fig. 2).

Postdive *f*H was significantly different after drug treatments compared with saline injection (Fig. 2). Postdive *f*H following saline injection ($309 \pm 25$ beats $\text{min}^{-1}$) increased to $392 \pm 22$ beats $\text{min}^{-1}$ after atropine injection (Fig. 2). Postdive *f*H decreased following injection of nadolol ($246 \pm 14$ beats $\text{min}^{-1}$), and a mixture of both atropine and nadolol resulted in a postdive *f*H of $333 \pm 16$ beats $\text{min}^{-1}$ (Fig. 2).

From the start to the end of the dive, *f*H increased progressively. For the controls, *f*H increased $50 \pm 13$ beats $\text{min}^{-1}$, which was significantly reduced by treatment with atropine as well as by a mixture of both atropine and nadolol. Removal of parasympathetic influences to the heart with atropine reduced the increase in *f*H during the dive to $14 \pm 3$ beats $\text{min}^{-1}$, and removal of both parasympathetic and sympathetic influences with a mixture of atropine and nadolol virtually eliminated any increase (the difference was only $1 \pm 4$ beats $\text{min}^{-1}$). Injection of nadolol reduced the *f*H increase to $30 \pm 6$ beats $\text{min}^{-1}$, although this value was not significantly different from the control value.

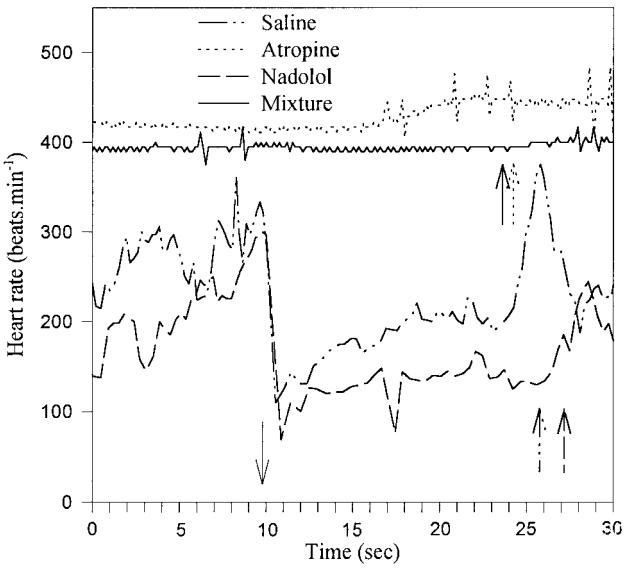


Figure 1. Heart rate (*f*H, beats $\text{min}^{-1}$) profiles of a duck voluntarily diving following injection of saline, atropine, nadolol, and a mixture of both atropine and nadolol treatments. *Down arrow*, indicates start of all dives. *Up arrow*, indicates completion of dive. The line style of the arrow indicating surfacing corresponds to the line style of *f*H profile.

An *f*H profile of the effect of isoproterenol is shown in Figure 3. The group mean average for routine *f*H increased significantly from $191 \pm 24$ beats $\text{min}^{-1}$ in the saline condition to $309 \pm 43$ beats $\text{min}^{-1}$ following stimulation of the sympathetic system by injecting isoproterenol (Fig. 4). Isoproterenol significantly increased *f*H during all phases of the dive cycle (Fig. 4). Predive *f*H ($338 \pm 1$ beats $\text{min}^{-1}$) after saline injection was increased with isoproterenol to $426 \pm 18$ beats $\text{min}^{-1}$ (Fig. 4).

Table 1: Mean dive times (s) after saline (control) or drug injection of dives selected for analysis of *f*H (±SEM)

| | Mean Dive Time (s) |
|---|---|
| Saline ($N = 5$) | 16 ± .8 |
| Atropine ($N = 5$) | 14 ± 1.5 |
| Nadolol ($N = 5$) | 15 ± .7 |
| Mix (atropine and nadolol) ($N = 5$) | 14 ± .9 |
| Saline ($N = 3$) | 16 ± 1.4 |
| Isoproterenol ($N = 3$) | 17 ± 2.2 |

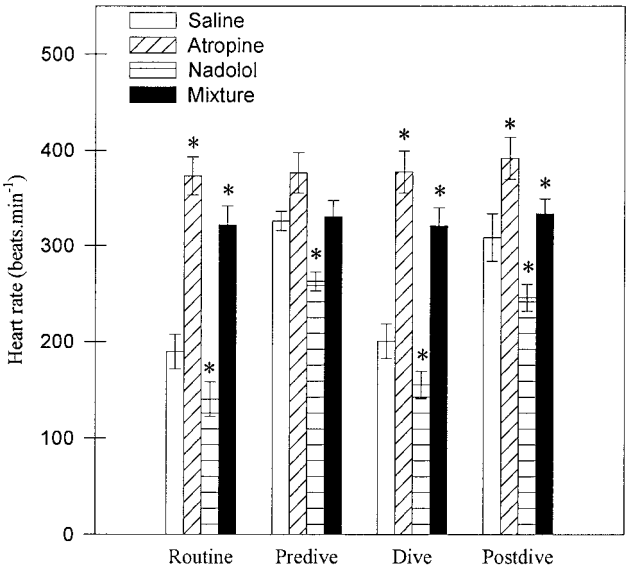


Figure 2. Effects of injected drugs on heart rate ($f$H, beats min$^{-1}$) of voluntarily diving ducks. Routine, predive, dive, and postdive $f$H values are shown for the saline, atropine, nadolol, and a mixture of both atropine and nadolol ($\pm$SEM, $N = 5$). An asterisk indicates $f$H values significantly different from the corresponding phase during the saline treatment.

$f$H during the dive increased after isoproterenol (245 $\pm$ 22 beats min$^{-1}$) from 207 $\pm$ 28 beats min$^{-1}$ in the controls (Fig. 4). Postdive $f$H increased from 296 $\pm$ 37 beats min$^{-1}$ to 399 $\pm$ 37 beats min$^{-1}$ in the saline and isoproterenol conditions, respectively (Fig. 4).

The difference in $f$H between control and isoproterenol treatment was determined for routine $f$H and $f$H during all phases of the dive cycle. The resulting increase in $f$H after isoproterenol during the dive was significantly lower than the increase in routine $f$H or that seen in the predive or postdive phases. Diving $f$H increased by 38 $\pm$ 8 beats min$^{-1}$ after isoproterenol, whereas the routine, predive, and postdive $f$H differences were 118 $\pm$ 20, 89 $\pm$ 18, and 102 $\pm$ 6 beats min$^{-1}$, respectively.

## Discussion

Our results demonstrate the dominance of the parasympathetic system in the control of $f$H during voluntary diving in ducks. The reduction in $f$H from the predive level in a voluntary dive is a result of an increase in vagal output and not due to withdrawal of sympathetic drive to the heart. Sympathetic outflow to the heart appears to be maintained throughout the dive because $f$H was even lower after injection of the $\beta$-adrenergic blocker nadolol compared with $f$H in control dives. Furthermore, the increase in diving $f$H following treatment with the $\beta$-adrenergic agonist isoproterenol indicates that the chronotropic response to sympathetic influences on the heart were not totally eliminated by the parasympathetic system during diving. Nevertheless, after isoproterenol treatment, the increase in diving $f$H was not as large as compared with the increase in routine $f$H or $f$H during the predive or postdive phase.

In contrast, in muskrats the parasympathetic system dominates all of the chronotropic effects of the sympathetic system to such a degree that isoproterenol injection appears without effect on diving $f$H (Signore and Jones 1995, 1996). This increase in vagal influence is due to accentuated antagonism between the two branches of the autonomic nervous system. Accentuated antagonism means that a large vagal output predominates in the control of $f$H and may totally or partially block the effects of $\beta$-adrenergic stimulation on the heart despite maintenance of sympathetic output (Levy 1971). Levy (1971) proposed two mechanisms for how this may be accomplished: (1) in response to a certain level of sympathetic stimulation, there is a cholinergically mediated reduction in the amount of norepinephrine released; and (2) the magnitude of the response to a certain level of sympathetic stimulation is cholinergically attenuated.

In ducks, however, the sympathetic influences are reduced but not eliminated. Therefore, it is possible that accentuated antagonism in ducks is partial and not total, being less than that in muskrats and more closely resembling that in dogs. There is a sympathetically mediated increase in $f$H in dogs exercising on a treadmill. However, the same level of vagal

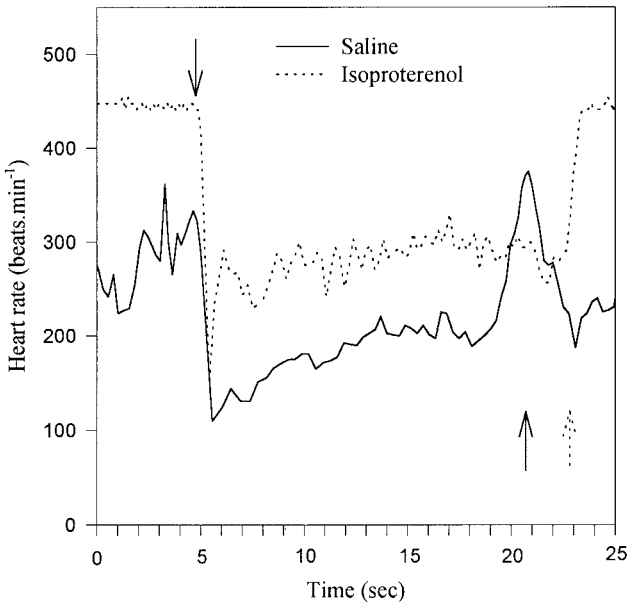


Figure 3. Heart rate ($f$H, beats min$^{-1}$) profiles of a duck voluntarily diving following saline and isoproterenol treatments. *Down arrow*, indicates start of all dives. *Up arrow*, indicates completion of dive. The line style of the arrow indicating surfacing corresponds to the line style of $f$H profile.

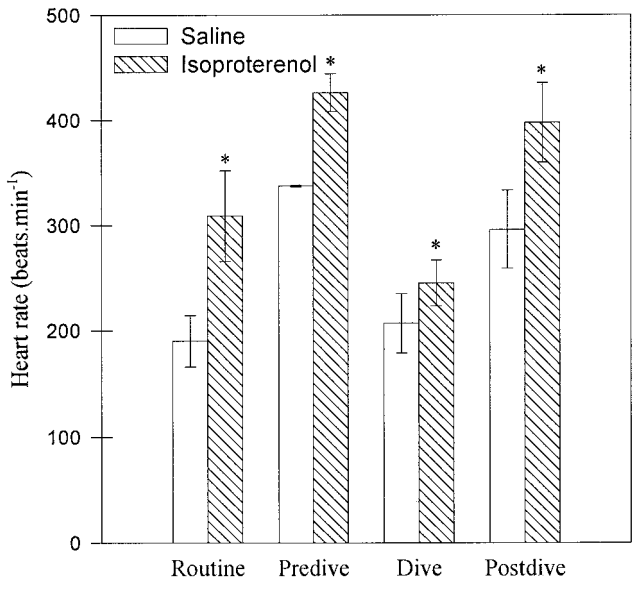


Figure 4. Effects of isoproterenol on heart rate ($f$H, beats min$^{-1}$) of voluntarily diving ducks. Routine, predive, dive, and postdive $f$H are shown for the saline and isoproterenol treatments ($\pm$SEM, $N = 3$). An asterisk indicates $f$H values significantly different from the corresponding phase during the saline treatment.

stimulation results in a greater negative chronotropic effect in dogs exercising than while at rest. Therefore, in spite of increased sympathetic activity, the response of the exercising dog's heart to parasympathetic influences is accentuated. (Stramba-Badiale et al. 1991). The most obvious reason for a lower level of accentuated antagonism in ducks is that vagal outflow is considerably less than in muskrats because diving $f$H in ducks is much higher than in muskrats. Even so, if sympathetic stimulation is artificially increased by isoproterenol, then accentuated antagonism at even a low level of vagal output is obvious. After isoproterenol, diving $f$H fell 181 $\pm$ 7 beats min$^{-1}$ from predive $f$H compared with 131 $\pm$ 28 beats min$^{-1}$ in the controls. In contrast, it is possible that the level of parasympathetic outflow to the heart was increased during a dive following isoproterenol treatment so that diving $f$H would be reduced compared with the increase in routine $f$H or $f$H during the predive or postdive phase, even in the absence of accentuated antagonism. A partial degree of accentuated antagonism in ducks, however, may be the explanation of the relationship observed between diving $f$H and predive $f$H during diving in ducks (Furilla and Jones 1987). Increases in $f$H in the predive phase, due to increased sympathetic activity, are present to a lesser extent during the dive (Furilla and Jones 1987). Therefore, diving $f$H will increase as predive $f$H increases, although the increase in diving $f$H will not be as great due to the partially accentuated antagonism between the parasympathetic and sympathetic nervous systems.

Sympathetic blockade with the $\beta$-adrenergic antagonist propranolol had no effect on diving $f$H in voluntarily diving ducks (Furilla and Jones 1987). The different effect on diving $f$H of the $\beta$-adrenergic blocker used in the present study, compared with that of Furilla and Jones (1987), may be a result of the different pharmacological effects of the two $\beta$-blockers, nadolol and propranolol. Propranolol readily crosses the blood-brain barrier in mammals because of its high lipid solubility compared with nadolol (Katzung 1992). In addition, propranolol has increased anesthetic properties compared with nadolol (Katzung 1992). The difference between propranolol and nadolol on $f$H during voluntary diving in muskrats has been clearly shown (Signore and Jones 1995). Propranolol-treated muskrats had an increased free-diving $f$H, whereas no effect on diving $f$H was seen using nadolol (Signore and Jones 1995). Also, muskrats treated with propranolol showed a reduced level of activity compared with those given nadolol (Signore and Jones 1995). Perhaps the lack of effect on $f$H during the dive with the $\beta$-blocker propranolol (Furilla and Jones 1987) may be due to a composite of the two effects of propranolol—as a $\beta$-blocker, reducing $f$H, and as an anesthetic, increasing $f$H.

The increase in $f$H that occurs during the dive following the initial $f$H reduction on submersion was significantly reduced, compared with controls, after atropine injection, whereas following injection of a mixture of both atropine and nadolol, this increase was virtually eliminated. Hence, this increase in $f$H during the dive appears to have two components, a withdrawal of parasympathetic activity and a contribution from increased sympathetic activation of the heart. This is further substantiated by noting that the sum of the increase following atropine (14 $\pm$ 3 beats min$^{-1}$) and after nadolol (30 $\pm$ 6 beats min$^{-1}$) is close to that of the controls (50 $\pm$ 13 beats min$^{-1}$).

A surprising aspect of this study was the duck's willingness to dive after treatment with the muscarinic blocker atropine. Ducks continued to dive despite having a diving $f$H in excess of 400 beats min$^{-1}$. In particular, one duck's behavior appeared totally unaffected by atropine, making 40 dives within 35 min, with a number of these dives in excess of 20 s. The effects of atropine on $f$H in this study were similar to those for muskrats (Signore and Jones 1995), yet one aspect remains puzzling in both studies. If predive $f$H is a composite of vagal withdrawal and sympathetic increase as indicated by the nadolol, atropine, and mixture treatments, why is there not an increase in predive $f$H from routine $f$H in the atropine treatment? It is evident that even in the presence of vagal tone, stimulation of the sympathetic system by isoproterenol increases predive $f$H above that seen with vagal blockade alone. A high dose of atropine in humans has been known to cause agitation (Katzung 1992). Agitation in ducks may increase the sympathetic drive to the heart during routine activity thereby elevating routine $f$H into a range normally associated with the predive phase.

**Acknowledgments**

This research was supported by grants to D.R.J. from the Natural Sciences and Engineering Research Council of Canada.

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