# Pharmacological blockade of the dive response: effects on heart rate and diving behaviour in the harbour seal (*Phoca vitulina*)

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## Summary

While diving, harbour seals (*Phoca vitulina*) manage their oxygen stores through cardiovascular adjustments, including bradycardia, a concurrent reduction in cardiac output, and peripheral vasoconstriction. At the surface, post-dive tachycardia facilitates rapid reloading of oxygen stores. Although harbour seals can tolerate >20 min of submergence, the majority of their natural dives are only 2–6 min and are usually followed by surface intervals that are <1 min, so they spend approximately 80% of their time submerged. Given that harbour seals meet their ecological needs through repetitive short aerobic dives, we were interested in the functional role, if any, of the dive response during these short dives. During voluntary diving in an 11 m deep tank, the cardiovascular responses to submergence of five harbour seals were manipulated using specific pharmacological antagonists, and the effects on diving behaviour were observed. Effects of pharmacological blockade on heart rate were also examined to assess the autonomic control of heart rate during voluntary diving. Heart rate was recorded using subcutaneous electrodes and data loggers, while diving behaviour was monitored using a video camera. The muscarinic blocker methoctramine blocked diving bradycardia, the α-adrenergic blocker prazosin blocked diving vasoconstriction, and the β-adrenergic blocker metoprolol blocked post-dive tachycardia. Heart-rate analysis indicated that diving bradycardia is primarily modulated by the vagus, while post-dive tachycardia results from parasympathetic withdrawal as well as increased sympathetic stimulation of the heart. None of the pharmacological blockers had any effect on average dive or surface interval duration. Seals maintained a high percentage of time spent diving in all treatments. Thus, harbour seals do not appear to need the dive response during short dives in order to maintain an efficient dive strategy.

Key words: diving, diving physiology, dive response, diving behaviour, heart rate, bradycardia, harbour seal, *Phoca vitulina*, methoctramine, metoprolol, prazosin, sympathetic system, parasympathetic system.

## Introduction

During diving, harbour seals rely on $O_2$ stores in their lungs, blood and muscles that are managed through cardiovascular adjustments, including bradycardia, a concurrent reduction in cardiac output, and peripheral vasoconstriction, collectively termed the 'dive response' (Irving et al., 1935; Scholander, 1940; Butler and Jones, 1997). Seals accomplish the majority of their ecological tasks underwater; therefore, a suitable diving strategy should minimize time at the surface and maximize the proportion of time spent underwater. Although harbour seals can tolerate >20 min of submergence (Harrison and Tomlinson, 1960; Eguchi and Harvey, 1995), the majority of their natural dives are only 2–6 min (Fedak et al., 1988; Eguchi and Harvey, 1995; Bowen et al., 1999). These routine dives are usually followed by brief surface intervals of <1 min duration so that foraging harbour seals spend 75–85% of their time at sea submerged (Fedak et al., 1988). In order to maintain a high percentage of dive time, seals must rely on aerobic-based metabolism during diving and restore blood gases rapidly when at the surface (Kooyman et al., 1980; Fedak, 1986). Seals balance their $O_2$ utilization with dive duration to avoid a significant anaerobic energy contribution to metabolism that usually prolongs post-dive recovery at the surface. After aerobic dives, surface intervals primarily function to reload $O_2$ stores and eliminate accumulated $CO_2$. Rapid restoration of blood gases between dives is facilitated by a high heart rate at the surface (post-dive tachycardia), which presumably reflects a high cardiac output and increased circulation to the peripheral tissues (Fedak, 1986; Butler and Jones, 1997).

While the cardiovascular responses to submergence are

clearly necessary during extended dives to conserve finite $O_2$ stores for the hypoxia-sensitive brain and heart, the role of these responses during routine diving is not as obvious. For instance, Signore and Jones (1995) found that in muskrats (*Ondatra zibethica*), when bradycardia and vasoconstriction were pharmacologically inhibited, maximum underwater survival time significantly decreased, yet the muskrats still dived voluntarily for periods that are as long as their routine dives. Furthermore, the cardiovascular responses to short dives are highly variable in seals. Jones et al. (1973) found that harbour seals did not always exhibit bradycardia during feeding dives that were <40 s. There is also evidence that bradycardia during short dives is not necessarily related to swimming speed or muscular work in seals (Kooyman and Campbell, 1972; Fedak, 1986; Williams et al., 1991).

Because it is unclear whether the cardiovascular components of the diving response are necessary during routine diving and, given that harbor seals meet their ecological needs through repetitive short aerobic dives, we were interested in the functional role, if any, of the dive response during short dives. In the present study, we used pharmacological blockers to investigate the necessity of diving bradycardia, vasoconstriction and surface tachycardia in the performance of short dives and short surface intervals in harbour seals. We also investigated whether these adjustments were necessary to maintain a high percentage of time spent underwater during diving bouts.

## Materials and methods
### Animals

Two adult (4-year-old) female and three juvenile (2-year-old) male harbour seals (*Phoca vitulina richardii* L.), ranging in mass from 40 kg to 60 kg, were held in freshwater pools at the University of British Columbia. The seals were maintained on a daily diet of herring supplemented with a vitamin (Mazuri Vita-Zu mammal tablet, PMI Nutrition International, Richmond, IN, USA).

### Instrumentation

Each seal was anaesthetized using 5% isoflurane (Janssen, Toronto, ON, Canada; induction by mask) and, after endotracheal intubation, the seal was maintained on 1–2% isoflurane and 98–99% $O_2$. Two electrocardiogram (ECG) electrodes were placed on the dorsal surface of the seal, one above the shoulder blade and one above the pelvis, on opposite sides of the animal. Hair was shaved from the areas where incisions were to be made, and the exposed skin was cleaned with 70% alcohol and an iodine-based solution (polyvinyl pyrolidine-iodine complex 10%, Iodovet, Rougier Pharma, Mirabel, QC, Canada). Thin-wire ECG electrodes (28 gauge, shielded, Cooner Wire Company, Chatsworth, CA, USA) were tunnelled subcutaneously 9 cm from the insertion site (one cranially and one caudally) with a 14 gauge hypodermic needle. Each ECG electrode was connected to an externalized waterproof lead and an underwater connector (USI square

miniconn, Underwater Systems, Stanton, CA, USA) that was glued to a neoprene base with 5-min epoxy (Devcon, Acklands, Vancouver, BC, Canada). After electrode insertion, the amplified ECG was displayed on an oscilloscope to verify that the electrode placement resulted in a clear signal. The underwater connector/neoprene base was then glued to the seal's hair using cyanoacrylate adhesive (ZapAGap, Richmond RC Supply Ltd, Delta, BC, Canada). The electrode insertion sites were bathed with 1 ml bupivacaine hydrochloride 25% (Abbott Laboratories Ltd, Montreal, QC, Canada) to provide post-operative analgesia. A colored neoprene patch was glued (ZapAGap) to the hair on each seal's head for identification on videotape. Two buckles were glued to the seal's hair mid-way between the two electrodes using 10-min epoxy (Evercoat Ten Set Epoxy; Fibreglass-Evercoat Co. Inc., Cincinnati, OH, USA) for the attachment of an ECG-recording instrument. Seals were allowed at least 48 h to recover before diving experiments. All procedures were approved by the Animal Care Committee at the University of British Columbia.

### Pharmacological antagonists

Preliminary experiments with three seals established the appropriate doses of the pharmacological blockers used in the diving experiments, as well as the time frame in which the drugs were most effective. Specific pharmacological agonists were used to induce the cardiovascular responses seen during diving in order to assess the doses of the blockers and the effectiveness of blockade. Before drug testing, a catheter [PE micro-renathane tubing, 0.050 units × 0.025 units o.d. × i.d. (Braintree Scientific Inc., Braintree, MA, USA) attached to a 21 gauge winged needle infusion set (Venisystems Abbott Laboratories Inc., Abbott Park, IL, USA)] was inserted into the extradural intravertebral vein under anaesthesia (see above protocol). The catheter was kept open by filling it with heparinized PVP [polyvinyl pyrolidine (Sigma-Aldrich Canada Ltd); 1 g PVP:12 ml saline, heparin 20 U ml$^{-1}$]. Each seal was restricted to a dry enclosure, the PVP was withdrawn from the catheter, and the catheter was attached to a saline-filled intravenous line (1.9 m; Interlink System, Baxter Corp., Toronto, ON, Canada). Heart rate was monitored after intravenous injection of each agonist alone (into the catheter extension), and then the effects of the agonists were monitored following administration of the appropriate antagonist. The β-adrenergic agonist isoproterenol hydrochloride (0.01 µg kg$^{-1}$; Sigma-Aldrich Canada Ltd) was used to induce tachycardia and therefore assess the efficacy of the $\beta_1$-adrenergic antagonist metoprolol (Novartis Pharmaceuticals Canada Inc., East Hanover, NJ, USA). The α–adrenergic agonist 1-phenylephrine hydrochloride (0.06 µg kg$^{-1}$; Sigma-Aldrich Canada Ltd) was used to induce both vasoconstriction and bradycardia in order to assess the efficacy of the $\alpha_1$-adrenergic antagonist prazosin (Pfizer Inc., New York, NY, USA) and the muscarinic antagonist methoctramine (Sigma-Aldrich Canada Ltd), respectively.

In one seal, several different doses of each blocker were tested for blockade of the agonist-induced response and for

unwanted side effects. The specific doses to be used in diving experiments were ultimately chosen based on the maximum drug dose causing the desired blockade (as indicated by heart-rate analysis) without any obvious side effects such as excitement or lethargy. These doses were then confirmed in two other seals. In all three seals, the selected doses were tested for blockade of the agonist-induced responses at different time intervals after administration of the antagonist (15 min to 2 h intervals for up to 6 h post dose). Based on these results, we estimated the time frame during which diving experiments would be conducted.

### *Diving experiments*

Diving experiments with five harbour seals were conducted in a 4.5 m × 11 m diameter × depth freshwater tank. Seals were allowed to acclimate to the tank over a period of 1–2 months. Water temperature ranged from 12°C to 16°C. All five seals received each of the following treatments, once, in randomized order: (1) subcutaneous (s.c.) injection of the cardio-selective muscarinic antagonist methoctramine ($0.23\,mg\,kg^{-1}$); (2) oral administration (in a fish) of the $\alpha_1$-adrenergic antagonist prazosin (three doses, $0.24\,mg\,kg^{-1}$ each); (3) oral administration of the $\beta_1$-adrenergic antagonist metoprolol (two doses, $4\,mg\,kg^{-1}$ each); (4) a combination of s.c. methoctramine and oral prazosin; (5) a combination of s.c. methoctramine and oral metoprolol; (6) s.c. injection of saline (control for all methoctramine injections); and (7) oral administration of a fish without pills (control for prazosin and metoprolol). Treatments were done on separate days with at least 24 h between drugs (48 h following metoprolol). Injections were given just before diving sessions while seals were at the surface platform of the dive tank. Oral pills (prazosin, metoprolol, or control fish) were given on the evening before and the morning of diving experiments.

Heart rate ($f_H$) was recorded using a custom-designed data logger that consisted of a high-memory ECG recorder based on a computer board (model 8; Onset Computer Corp., Bourne, MA, USA) interfaced to a compact-flash memory expansion board (model CF8; Peripheral Issues, Mashpee, MA, USA) (for details, see Andrews, 1998; Southwood et al., 1999). The data logger was programmed to sample the amplified ECG signal at 50 Hz and, with a memory of 15 Mb, recorded $f_H$ for 84 h. Before diving sessions, the data logger was attached to the buckles on the seal and connected to the ECG electrodes *via* underwater connectors. During experiments, voluntary diving behaviour was recorded using a video camera (Lorex, Strategic Vista Corp., Markham, ON, Canada) suspended over the breathing hole ($2.4\,m^2$) in which the seals surfaced.

### *Statistics and analysis*

Data were downloaded to a computer from the data logger, and inter-beat intervals were calculated by detecting the R waves of the ventricular QRS complexes of the ECG. Instantaneous heart rate was determined by converting R–R intervals to beats min$^{-1}$, and mean $f_H$ for dives and surface intervals was calculated by averaging these values. The first and last 10 s of each dive and the last 3 s of each surface interval were excluded from the calculation of means to reduce variability in $f_H$ caused by the initial bradycardia that is below the $f_H$ established during the rest of the dive, cardiac acceleration before surfacing (anticipatory tachycardia), and cardiac deceleration before submergence (anticipatory bradycardia). Therefore, only dives of >20 s and surface intervals of >3 s were analyzed. For each treatment, diving behaviour (dive and surface interval durations) was analyzed from the videotapes for the hour during which the blockade was maximal. This hour of analysis was initially estimated during preliminary drug testing and ultimately determined by analysis of $f_H$ during diving sessions. For methoctramine-treated groups, dive behaviour was analyzed approximately 1–2 h after injections; for prazosin, 1.25–2.25 h after the third oral dose; and for metoprolol, 4–5 h after the second oral dose. Controls for each group were analyzed to match these time periods. Values for $f_H$ and dive behaviour given in the text represent grand means ± S.E.M. ($N$=5) for each treatment.

The means for each group were compared using one-way repeated measures analysis of variance (ANOVA). Multiple comparisons were performed using Tukey tests. Differences were considered significant when $P<0.05$. All statistics were calculated with SigmaStat software (Jandel Scientific, San Rafael, CA, USA).

### Results

During preliminary drug testing, the $\alpha$-adrenergic agonist 1-phenylephrine hydrochloride was used to assess the efficacy of the muscarinic antagonist methoctramine and the $\alpha_1$-adrenergic antagonist prazosin. Phenylephrine ($0.06\,\mu g\,kg^{-1}$ i.v.) alone caused an 80% reduction in mean $f_H$ (Fig. 1A,B) and instantaneous heart rates as low as 9 beats min$^{-1}$. This vagally mediated bradycardia is a barostatic reflex resulting from phenylephrine-induced vasoconstriction raising arterial blood pressure. Subcutaneous injection of methoctramine ($0.23\,mg\,kg^{-1}$ s.c.) blocked this response so that phenylephrine decreased $f_H$ only by approximately 16% 0.5 h after methoctramine injection (Fig. 1A). Following oral administration of prazosin ($0.24\,mg\,kg^{-1}$), phenylephrine only caused a slight decrease in $f_H$ (29% decrease 1.25 h after oral dose) compared with phenylephrine alone, and this was probably a result of $\alpha$-adrenergic blockade of vasoconstriction (Fig. 1B). The $\beta$-adrenergic agonist isoproterenol hydrochloride was used to assess the dose and effectiveness of the $\beta_1$-adrenergic antagonist metoprolol. Isoproterenol ($0.01\,\mu g\,kg^{-1}$ i.v.) alone caused $f_H$ to increase by 127% before $\beta$-adrenergic blockade with metoprolol but only by 53% 3.5 h after metoprolol administration ($4\,mg\,kg^{-1}$ oral) (Fig. 1C).

The effects of the pharmacological blockers on dive and post-dive surface interval $f_H$ are presented in Fig. 2, and diving $f_H$ profiles are shown in Fig. 3. In the control groups, mean dive $f_H$ ranged from 47±3 beats min$^{-1}$ to 49±4 beats min$^{-1}$, and mean surface interval $f_H$ ranged from 133±3 beats min$^{-1}$ to

138±4 beats min$^{-1}$ (Fig. 2). During a typical dive bout in control seals, $f_H$ dropped immediately upon diving to approximately 17% of the pre-dive surface rate within 5–10 s of the dive. $f_H$ then increased to approximately 35% of the pre-dive rate within 30–40 s of the initiation of the dive and remained at this level until approximately 10–20 s before
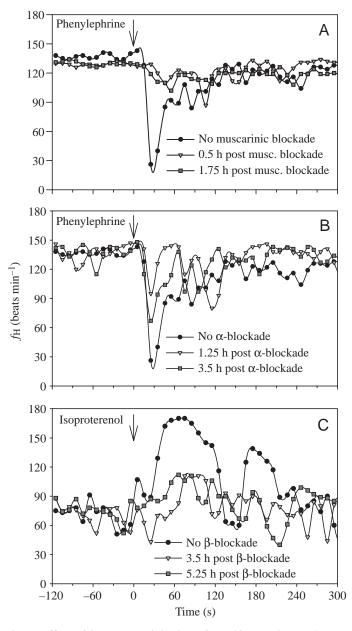
surfacing, when it increased rapidly so that pre-dive levels were reached upon or within 5 s of surfacing (Fig. 3). In the α- and β-adrenergic-blocked groups, the $f_H$ profiles followed a similar pattern to those in the control group (an initial drop, a slight increase to a steady level and then a pre-surfacing increase to surface levels). In the three muscarinic-injected groups, $f_H$ decreased to a lesser degree such that the extreme initial drop and steep increase 10–20 s before surfacing were not pronounced (Fig. 3).

In the muscarinic-blocked group, mean dive $f_H$ was significantly higher ($P<0.001$, $N=5$) than in the control group (110±3 beats min$^{-1}$ versus 49±4 beats min$^{-1}$), while mean surface $f_H$ was not significantly different from the control group (137±3 beats min$^{-1}$ versus 138±4 beats min$^{-1}$) (Fig. 2). Dive $f_H$ in α-adrenergic-blocked animals was significantly higher ($P<0.001$, $N=5$) than in control seals (64±3 beats min$^{-1}$ versus 47±3 beats min$^{-1}$), but surface rates were not significantly different (121±5 beats min$^{-1}$ versus 133±3 beats min$^{-1}$) (Fig. 2). After β-adrenergic blockade, dive $f_H$ was not significantly different from that of the control group (42±3 beats min$^{-1}$ versus 48±3 beats min$^{-1}$), but surface $f_H$ was



Fig. 1. Effect of intravenous injection of specific agonists on heart rate ($f_H$) before and after blockade with the appropriate antagonist in one seal. Each data point represents the mean $f_H$ for the preceding 10 s interval. Arrows denote the time of injection of agonists. (A) 1-phenylephrine hydrochloride (0.06 µg kg$^{-1}$) alone and after s.c. injection of the muscarinic antagonist methoctramine (0.23 mg kg$^{-1}$). (B) Phenylephrine (0.06 µg kg$^{-1}$) alone and after oral administration of the α$_1$-adrenergic antagonist prazosin (0.24 mg kg$^{-1}$). (C) Isoproterenol hydrochloride (0.01 µg kg$^{-1}$) alone and after the oral administration of β$_1$-adrenergic antagonist metoprolol (4 mg kg$^{-1}$). Control saline injections caused no significant effect on $f_H$.
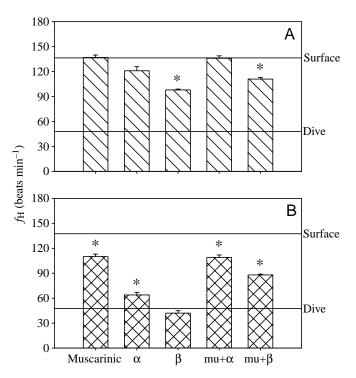


Fig. 2. Mean heart rate ($f_H$) ± S.E.M. during (A) post-dive surface intervals and (B) dives in control and pharmacologically blocked harbour seals ($N=5$). Heart rate data from control groups were combined to give the mean $f_H$ indicated by the horizontal lines, although statistical analyses were performed on controls for each data set. * indicates values significantly different from the control group within dive states ($P<0.05$). For each treatment, the dive $f_H$ was significantly lower than the surface interval $f_H$. α, seals treated with α-adrenergic blocker; β, seals treated with β-adrenergic blocker; mu+α, seals treated with muscarinic and α-adrenergic blockers combined; mu+β, seals treated with muscarinic and β-adrenergic blockers combined.

significantly lower ($P<0.001$, $N=5$) ($98\pm1$ beats min$^{-1}$ *versus* $137\pm3$ beats min$^{-1}$) (Fig. 2). In the muscarinic- plus $\alpha$-adrenergic-blocked group, dive $f_H$ was significantly higher ($P<0.001$, $N=5$) than in control seals ($109\pm3$ beats min$^{-1}$ *versus* $49\pm4$ beats min$^{-1}$), but surface rates were not significantly different ($136\pm3$ beats min$^{-1}$ *versus* $133\pm3$ beats min$^{-1}$) (Fig. 2). Dive $f_H$ after muscarinic- plus $\beta$-adrenergic blockade was significantly higher ($P<0.001$, $N=5$) than in the control ($88\pm1$ beats min$^{-1}$ *versus* $49\pm4$ beats min$^{-1}$), and surface $f_H$ was also significantly lower ($P<0.001$, $N=5$) ($111\pm2$ beats min$^{-1}$ *versus* $138\pm4$ beats min$^{-1}$) (Fig. 2). In each treatment condition, the dive $f_H$ was significantly lower ($P<0.001$, $N=5$) than the surface $f_H$ (Fig. 2).

Fig. 4 shows the effect of pharmacological blockade on mean dive and post-dive surface interval duration. Mean

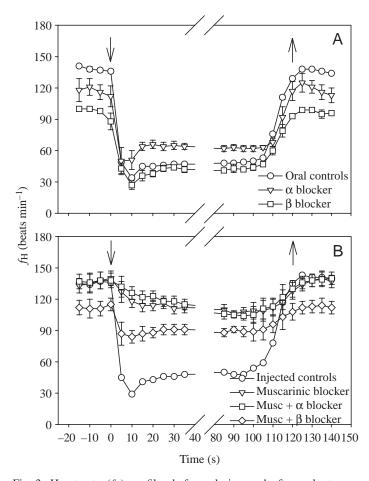dive duration in control seals ranged from $2.61\pm0.32$ min to $2.83\pm0.49$ min, and mean surface-interval duration ranged from $0.40\pm0.04$ min to $0.43\pm0.04$ min (Fig. 4). None of the treatments had any significant effect on mean dive duration ($2.34\pm0.47$ min for the muscarinic group; $2.40\pm0.27$ min for the $\alpha$-adrenergic group; $2.80\pm0.39$ min for the $\beta$-adrenergic group; $2.67\pm0.45$ min for the muscarinic plus $\alpha$-adrenergic group; $2.67\pm0.47$ min for the muscarinic plus $\beta$-adrenergic group; Fig. 4). In fact, seals made voluntary dives for as long as 8.12 min without a surface tachycardia, 6.72 min when bradycardia was blocked, 4.72 min when vasoconstriction was blocked, and 4.93 min when both bradycardia and vasoconstriction were blocked. Furthermore, there was no significant change in mean surface interval duration after blockade ($0.40\pm0.04$ min for the muscarinic group; $0.47\pm0.04$ min for the $\alpha$-adrenergic group; $0.44\pm0.02$ min for the $\beta$-adrenergic group; $0.48\pm0.02$ min for the muscarinic plus $\alpha$-adrenergic group; $0.44\pm0.04$ min for the muscarinic plus $\beta$-adrenergic group; Fig. 4).

There was no effect of blockade on the percentage of time spent submerged during diving sessions. In control seals, mean percentage dive time ranged from $86\pm1\%$ to $87\pm1\%$, and in treated seals, percentage dive time ranged from $83\pm2\%$ to $85\pm2\%$.
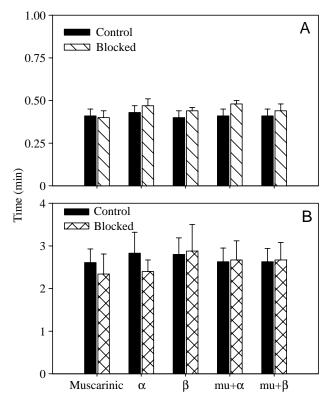
Fig. 3. Heart rate ($f_H$) profiles before, during and after voluntary dives in (A) $\alpha$-adrenergic- and $\beta$-adrenergic-blocked harbour seals and in (B) muscarinic-, muscarinic- plus $\alpha$-adrenergic-, and muscarinic- plus $\beta$-adrenergic-blocked seals. Control data for the two oral drugs (A) and for the three injected groups (B) were combined, although statistical analyses were performed on controls for each data set. Arrows denote the beginning and end of the dive. Each data point represents the mean $f_H$ for the preceding 5 s interval. For each treatment, mean $f_H$ during two dives (approximately 120 s) were averaged for each animal. Data from all seals ($N=5$) were then combined to give the means $\pm$ S.E.M. illustrated. The data were normalized so that dives of different lengths ended at the same time.

Fig. 4. (A) Mean surface interval and (B) dive durations $\pm$ S.E.M. in control and pharmacologically blocked harbour seals ($N=5$). Note that the time scales for each graph are different. $\alpha$, seals treated with $\alpha$-adrenergic blocker; $\beta$, seals treated with $\beta$-adrenergic blocker; mu+$\alpha$, seals treated with muscarinic and $\alpha$-adrenergic blockers combined; mu+$\beta$, seals treated with muscarinic and $\beta$-adrenergic blockers combined.

## Discussion

Diving induced a marked decrease in $f_H$ to approximately 35% of the surface level in control seals. Previous studies have also shown a similar drop in $f_H$, from 25% to 50% of surface levels, during voluntary dives in seals (Påsche and Krog, 1980; Jones et al., 1973; Fedak et al., 1988). Diving bradycardia was present in all control dives regardless of dive duration. In fact, seals displayed bradycardia even when they dipped their heads under water for periods of <20 s. This is in contrast to the findings of Jones et al. (1973) who showed that one harbour seal did not display a bradycardia during some short feeding dives. Post-dive tachycardia was present during all control surface intervals regardless of surface interval duration. Resting $f_H$ was not formally recorded from these seals during diving sessions because diving was continuous, but, during periods of rest on land in preliminary experiments, $f_H$ was approximately 75 beats min$^{-1}$. In control seals, diving $f_H$ was always below this level, and surface $f_H$ was always well above it.

The effects of the blockers on $f_H$ were in agreement with their pharmacological action on the autonomic nervous system. Methoctramine is a polymethylene tetra-amine compound that is highly selective for $M_2$-subtype muscarinic receptors that are predominantly found in the heart in many terrestrial mammals (Hammer and Giachetti, 1982; Giraldo et al., 1988; Melchiorre, 1988; Hendrix and Robinson, 1997). Therefore, methoctramine reduced diving bradycardia by inhibiting the action of acetylcholine on cardiac $M_2$ receptors in the seals. Prazosin is a highly selective $\alpha_1$-adrenergic antagonist with an affinity for $\alpha_1$ receptors that is approximately 1000-fold greater than for $\alpha_2$ receptors (Davey, 1980; Hoffman, 2001). In humans and other terrestrial mammals, blockade of $\alpha_1$ receptors inhibits vasoconstriction induced by catecholamines so that vasodilation occurs in arterioles. The fall in peripheral vascular resistance leads to decreases in arterial blood pressure and, as a result of the barostatic reflex, slight increases in $f_H$ and cardiac output (Davey, 1980; Saeed et al., 1982; Hoffman, 2001). Prazosin caused a slight but significant increase in diving $f_H$, probably as a result of $\alpha$-adrenergic blockade causing peripheral vasodilation. Although we could not monitor blood flow and arterial blood pressure during dives, the increase in $f_H$ after administration of prazosin, as well as the lack of a marked effect of the $\alpha$-adrenergic agonist phenylephrine in prazosin-treated animals, suggests that $\alpha$-adrenergic blockade was indeed effective in our seals. Metoprolol is a $\beta_1$-selective adrenergic antagonist that blocks the action of noradrenaline on $\beta_1$ receptors that are predominantly found in the myocardium in humans (Prichard and Tomlinson, 1986; Hoffman, 2001). Therefore, metoprolol inhibited post-dive surface tachycardia by blocking sympathetic inputs to $\beta$-adrenergic receptors on the heart.

The effects of cardiovascular pharmacological blockade reveal the dynamic influence on $f_H$ of the two branches of the autonomic nervous system during diving. Because mean surface $f_H$ was unchanged by muscarinic blockade but significantly lower after $\beta$-adrenergic blockade, post-dive tachycardia is attributed to increased sympathetic stimulation of the heart, as well as vagal withdrawal at the surface. Mean dive $f_H$ after muscarinic blockade was significantly higher than the dive $f_H$ in control seals, whereas dive $f_H$ following $\beta$-adrenergic blockade was not significantly different; therefore, the parasympathetic nervous system is the primary modulator of bradycardia during diving. However, the role of the sympathetic system during diving is not as straightforward. The $f_H$ during dives was significantly lower than during surface intervals in muscarinic-blocked seals, suggesting that an increased level of sympathetic stimulation at the surface is withdrawn during submergence. Sympathetic inputs to the heart cannot be withdrawn completely though, because diving $f_H$ after $\beta$-adrenergic plus muscarinic blockade was significantly lower than after muscarinic blockade alone. However, $\beta$-adrenergic blockade alone did not significantly lower diving $f_H$.

One possible explanation for these discrepancies in diving $f_H$ is that the two divisions of the autonomic nervous system interact asymmetrically such that the parasympathetic system dominates the sympathetic system when vagal outflow to the heart is maximal. In other words, sympathetic tone persists during diving but is not expressed because the vagus modulates $f_H$ by means of an accentuated antagonism. Accentuated antagonism has also been observed in diving muskrats (Signore and Jones, 1995) and is the result of a cholinergically mediated insensitivity of cardiac cells to adrenergic stimulation (Kimura et al., 1985; Signore and Jones, 1995). Such a response during diving would explain why harbour seals develop a bradycardia despite increases in circulating catecholamines (Hance et al., 1982; Hochachka et al., 1995). It would also facilitate the rapid switching between dive and surface states, because the effective response to changes in sympathetic activation occurs more slowly than changes resulting from parasympathetic activity (Furilla and Jones, 1987; Japundzic et al., 1990).

A puzzling result is that the dive $f_H$ was significantly lower than the surface $f_H$ in muscarinic- plus $\beta$-adrenergic-blocked seals. Simultaneous blockade of parasympathetic and sympathetic outflow to the heart should reveal the aneural or intrinsic $f_H$. In our double-blocked seals, dive $f_H$ was 88 beats min$^{-1}$, whereas surface $f_H$ was 111 beats min$^{-1}$. This finding suggests that either blockade was not complete or that there is a non-muscarinic, non-$\beta_1$-adrenergic factor affecting $f_H$ during diving in harbour seals. One possibility is that the surface tachycardia may be caused by stimulation of cardiac $\beta_2$ receptors by circulating adrenaline. We chose a $\beta_1$-selective antagonist in order to avoid effects on $\beta_2$-adrenergic receptors in vascular and bronchial smooth muscle. Also, sympathetic stimulation of the heart in humans is known to occur primarily via $\beta_1$ receptors, although it is uncertain as to what extent activation of cardiac $\beta_2$ receptors contributes to increases in $f_H$ (Hoffman, 2001). It is likely that $\beta_2$ receptors play a larger role in cardiac responses in seals.

On several occasions, seals displayed a decrease in $f_H$ 1–3 s before submergence. Anticipatory bradycardia has previously

been observed in harbour seals (Jones et al., 1973). Furthermore, pre-surfacing tachycardia was seen in all control dives and was unaffected by α- or β-adrenergic blockade but was reduced in methoctramine-injected animals, which suggests that it is caused by the withdrawal of vagal inputs. Cardiac acceleration before surfacing has been reported in both seals (Jones et al., 1973; Thompson and Fedak, 1993; Andrews et al., 1997) and muskrats (Signore and Jones, 1995). By restoring circulation to tissues that may have been hypoperfused during the dive, pre-surfacing tachycardia should further reduce the $O_2$ content of the blood, thereby maximizing $O_2$ uptake at the beginning of the surface interval (Thompson and Fedak, 1993).

Previous studies reveal that harbour seals in the wild typically dive for 2–6 min, with surface intervals lasting <1 min, so they spend 75–85% of their time at sea submerged (Fedak et al., 1988; Eguchi and Harvey, 1995; Bowen et al., 1999). Our data agree with literature values. In control seals, dive duration ranged from 23 s to 5.4 min, and the mean duration was 2.7 min; surface intervals ranged from 4 s to 1.4 min, and mean surface-interval duration was 25 s. During control diving sessions, seals spent 86% of their time submerged.

Pharmacological blockade of diving bradycardia, vasoconstriction and post-dive tachycardia did not significantly affect routine dive or surface-interval duration. Evidently, our seals had enough onboard $O_2$ to maintain routine dives without the $O_2$-conserving dive response and also to prevent an $O_2$ debt large enough to require extra time at the surface. For Weddell seals (*Leptonycotes weddellii*), dives that involve an increasing reliance on anaerobic metabolism usually necessitate extended surface intervals to replenish glycolytic fuel reserves, process anaerobic byproducts, and restore blood and tissue pH (Kooyman et al., 1980). Although we did not measure post-dive blood lactate levels, the seals did not surface or haul out on the deck for extended recovery periods, so it is likely that they avoided significant anaerobic energy contributions to diving metabolism. Furthermore, assuming that β-blocked seals did not fully reload their $O_2$ stores at the surface, their $O_2$ reservoir was still large enough to enable continuous diving (and some dives as long as 8.1 min). Seals also maintained a high percentage dive time (approximately 84%) in all treatments; thus, the cardiovascular dive response was not necessary to maintain an 'efficient' dive strategy during short diving sessions.

The short dives made by our seals in the control and treatment groups were all within estimates of their aerobic dive limit (ADL). This limit is defined as the maximum amount of time a diver can remain submerged relying only on aerobic biochemical pathways (Kooyman et al., 1983). The ADL can be empirically determined by measuring post-dive blood lactate, the main metabolite of anaerobiosis, or an estimate of the ADL (cADL) can be calculated using the quotient of estimated values for $O_2$ stores and diving metabolic rate (DMR). Specifically, if total body $O_2$ stores in the harbour seal equal 57 ml kg$^{-1}$ (assuming 50% desaturation of arterial blood

and 85% desaturation of venous blood; Davis et al., 1991), and if the DMR is equal to the resting metabolic rate (RMR) of 7.3 ml $O_2$ min$^{-1}$ kg$^{-1}$ (Davis et al., 1991), then the cADL should be 7.8 min. In fact, RMR is essentially the metabolic rate when no $O_2$-conserving mechanisms are being utilized; therefore, harbour seals are theoretically capable of diving for up to 7.8 min without the dive response (if they use all of their available $O_2$ stores). It follows that any $O_2$-conserving mechanism could potentially increase this aerobic limit, or, alternatively, any physiological response resulting in higher $O_2$ demands such as exercise or stress could potentially decrease it. Although we did not measure DMR in this study, the activity level of the seals during diving experiments was probably quite low compared with that of seals foraging in nature. On the other hand, Davis et al. (1985) showed that harbour seals swimming in a flume at 1.4 m s$^{-1}$ increased their $O_2$ consumption two times above the resting rate. Even if the DMR is equal to twice the RMR, the cADL should be 3.9 min. Because mean dive durations in control and treated seals ranged from 2.3 min to 2.8 min, all dives were probably aerobic in nature.

In a similar study, Signore and Jones (1995) found that, after pharmacological blockade of the dive response, muskrats still dived voluntarily for periods as long as their cADL, but maximum underwater survival time significantly decreased. Although we did not measure maximum underwater survival times in our seals, we expect that blockade of the dive response should limit dive duration and also extend recovery time at the surface for dives beyond the cADL. Again, if seals are capable of diving for up to 7.8 min without any $O_2$-conserving mechanisms (depending on the DMR), then it follows that any dives beyond that limit would either require some degree of a cardiovascular dive response and some degree of metabolic suppression or, alternatively, an increasing reliance upon anaerobic metabolism to meet energy demands.

If estimates of the ADL are in fact correct, then the seals in this study, and perhaps seals in the wild, often surface before they reach their aerobic limits. Why not remain submerged until $O_2$ stores are nearly exhausted? Optimality models have been used to tackle this question, and factors that limit time at the surface and thus the extent of preparation for a subsequent dive may limit dive duration. Such factors might include increased predation risk while at the surface or a reoxygenation rate that declines with surface interval time so that $O_2$ is gained with diminishing returns (Kramer, 1988; Houston and Carbone, 1992). Based on breath-by-breath measurements of end-tidal $O_2$ and $CO_2$ concentrations during surface intervals in harbour porpoises (*Phocoena phocoena*) and grey seals (*Halichoerus grypus*), Boutilier et al. (2001) recently proposed that surface-interval duration is governed by the readjustment of $CO_2$ stores rather than $O_2$ stores. Perhaps the accumulation of $CO_2$ and the resulting increase in tissue and blood pH could dictate the end to an aerobic dive. Although seals can tolerate much higher arterial $CO_2$ tensions compared with terrestrial mammals (Kerem and Elsner, 1973), a study of harp (*Pagophilus groenlandicus*) and hooded seals (*Cystophora cristata*) indeed

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showed that dive duration decreased significantly with increasing alveolar $CO_2$ tension (Påsche, 1976).

Although the harbour seals in this study could perform a series of short aerobic dives without the cardiovascular dive response, control seals consistently displayed a cardiac response during routine diving, suggesting that bradycardia has some utility. A relatively moderate degree of bradycardia and peripheral vasoconstriction is probably utilized during such short dives to limit the depletion of blood $O_2$ by peripheral organs and particularly by the muscles, thereby reserving $O_2$ stores for the brain and heart in case of emergencies (i.e. unplanned extension of submergence). While some supplementation of the muscle $O_2$ store could delay the onset of anaerobic metabolism (Davis and Kanatous, 1999; Jobsis et al., 2001), unrestricted blood flow to the muscles would limit aerobic dive capacity. Because of the greater affinity of myoglobin for $O_2$ compared with haemoglobin, blood-borne $O_2$ would quickly diffuse into the active muscles and render the local myoglobin-bound $O_2$ store unavailable for use. Davis and Kanatous (1999) developed a numerical model that describes the potential importance of the dive response in optimizing the use of blood and muscle $O_2$ stores during dives involving different levels of muscular exertion. They found that blood and muscle $O_2$ stores should be consumed simultaneously but that cardiac output and muscle perfusion must be reduced below resting levels in order to maximize the ADL over a range of diving metabolic rates ($2–9\,ml\,O_2\,min^{-1}\,kg^{-1}$). Furthermore, Jobsis et al. (2001) found that during trained submersions of harbour seals, increased muscle blood flow was accompanied by a reduction in myoglobin desaturation, suggesting a higher rate of $O_2$ extraction from the blood even though muscle perfusion during submersion was significantly reduced from resting values.

Although post-dive tachycardia was also not necessary to sustain a series of short aerobic dives punctuated by short surface intervals, control seals consistently displayed high heart rates at the surface. In between short dives, surface tachycardia facilitates the restoration of blood gases and $O_2$ stores to pre-dive levels (Thompson and Fedak, 1993; Andrews et al., 1997). While seals are able to dive continuously without this degree of tachycardia, diving with a larger reservoir of $O_2$ would allow for greater flexibility in behaviour in that a 'safety margin' would be available if the dive must be extended.

In conclusion, our data indicate that harbour seals are able to maintain routine dive and post-dive surface-interval durations as well as a high percentage of time underwater when the $O_2$-conserving dive response is pharmacologically inhibited. Nevertheless, our seals utilized the response during all control dives, regardless of dive duration. While they may not be necessary, cardiovascular adjustments are probably utilized during short dives in order to maximize aerobic dive capacity and to conserve $O_2$ for emergencies. This study raises some fundamental questions as to why seals surface before they reach their ADL and also what the functional role of the dive response is during short routine dives.

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