The Journal of Experimental Biology **198**, 2307–2315 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

EFFECT OF PHARMACOLOGICAL BLOCKADE ON CARDIOVASCULAR RESPONSES TO VOLUNTARY AND FORCED DIVING IN MUSKRATS

PIERRE E. SIGNORE AND DAVID R. JONES

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada V6T 1Z4

Accepted 18 July 1995

Summary

Neural control of free and forced diving bradycardia and peripheral resistance was studied in the muskrat (*Ondatra zibethicus*) by means of acute pharmacological blockade with the muscarinic blocker atropine, the α-adrenergic blocker phentolamine and the β-adrenergic blockers nadolol and propranolol. Saline injection was used as a control. Heart rate in control animals increased before voluntary dives and dropped markedly as soon as the animals submerged. Heart rate started increasing towards the end of voluntary dives and reached pre-dive values within the first 5 s of recovery. Pre-dive and post-dive tachycardia were reduced in β-blocked animals, emphasizing the role of the sympathetic system during the preparatory and recovery periods of voluntary dives. Diving bradycardia and the acceleration in heart rate before surfacing were abolished by atropine and unaffected by nadolol, demonstrating the importance of vagal efferent activity during diving. The results after blockade with nadolol suggest that there is an accentuated antagonism between the two branches of the autonomic nervous system during diving, so that parasympathetic influences on the heart predominate. Propranolol-treated muskrats had a higher diving heart rate than saline- and nadolol-treated animals, which may be due to a sedative effect caused by propranolol crossing the blood–brain barrier, a blockade of central catecholaminergic pathways or a peripheral neural effect, due to the anaesthetic properties of propranolol. Phentolamine did not affect diving bradycardia, indicating that diving bradycardia occurs independently of peripheral vasoconstriction. Compared with voluntary dives, heart rate was lower before forced dives in unrestrained animals and fell to lower values during diving. Underwater endurance in forced dives was markedly reduced after atropine and phentolamine treatment (either individually or combined) compared with that of control animals. Hence, bradycardia and peripheral vasoconstriction are necessary for maximum underwater endurance. A puzzling finding was that animals without bradycardia and/or peripheral vasoconstriction still dived voluntarily for periods as long as the calculated aerobic dive limit of muskrats and survived 5 min of forcible submergence. We hypothesize that anaerobic metabolism may play an important role during diving in muskrats.

Key words: diving bradycardia, aerobic dive limit, muskrat, *Ondatra zibethicus*, telemetry, atropine, nadolol, propranolol, phentolamine, sympathetic system, parasympathetic system.

Introduction

Cardiovascular changes evoked by forced submergence of birds and mammals have been extensively studied for more than a century (Bert, 1870; Richet, 1894). These changes include a vagally mediated bradycardia and an increase in peripheral vascular resistance brought about by the adrenergic system. These adjustments are presumed to be largely reflexogenic and result from a set of complex interactions (Daly, 1984; Jones *et al.* 1988). Input from nasal receptors initiates the response to forced submergence in mammals and diving ducks (Drummond and Jones, 1979; Dykes, 1974; Furilla and Jones, 1986*a*), whereas chemoreceptor drive initiates the response in dabbling ducks (Jones *et al.* 1982). The baroreflex also seems to be reset during forced dives (Daly, 1984; Smith and Jones, 1992) and apnoea is necessary for the response to occur (Daly, 1984; Drummond and Jones, 1979). More recent studies have shown that the diving response is also present in voluntarily diving animals but that it is much more labile (Jones *et al.* 1988). Observations such as cardiac deceleration and acceleration in anticipation of submersion and surfacing, respectively (Jones *et al.* 1973), habituation of diving bradycardia (Gabbot and Jones, 1987) and conditioning of bradycardia (Ridgway *et al.* 1975) suggest that the free-diving response may be controlled by higher centres within the central nervous system. Vagal outflow to the heart plays a major role in free-diving bradycardia (MacArthur and Karpan, 1989; Murdaugh *et al.* 1961), while β-blockade has little effect

on diving bradycardia in ducks (Furilla and Jones, 1987). However, a systematic study of the autonomic control of heart rate during voluntary diving and its effects on underwater endurance has not been carried out. In the present study, we investigated the autonomic nervous pathways involved in the free-diving response using pharmacological blockers. We acutely treated muskrats (*Ondatra zibethicus*) with the muscarinic blocker atropine, the α-adrenergic blocker phentolamine and the β-adrenergic blockers nadolol and propranolol, and studied, using telemetry, the effects of drugs on heart rate during voluntary dives. We also studied unrestrained forced dives for comparison. Finally, we investigated the effects of these drugs on voluntary diving performance as well as on maximum underwater endurance in forced dives.

Materials and methods

Five adult muskrats (three males and two females), ranging in mass from 0.8 to 1.0 kg, were used in 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, Missouri) 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). 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, California) 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, 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.

All muskrats received the following treatments in randomized order: untreated (i.e. no injection), injection of saline (control) or injection of the muscarinic antagonist atropine sulphate (1 mg kg^{-1}, Sigma, St Louis, Missouri), the α-adrenergic antagonist phentolamine mesylate (1 mg kg^{-1}, Rogitine, Ciba-Geigy, Mississauga, Ontario) or the β-adrenergic antagonists DL-propranolol hydrochloride (4 mg kg^{-1}, Sigma) and nadolol (4 mg kg^{-1}, Sigma). In addition, a mixture of atropine (1 mg kg^{-1}) and phentolamine (1 mg kg^{-1}) was administered as the final treatment in all cases. Drugs were mixed in 0.9 % saline and injected subcutaneously at the beginning of diving sessions. Diving sessions for untreated, saline, propranolol, nadolol and atropine groups lasted 2 h, and sessions for phentolamine and atropine–phentolamine groups were for 1 h because of the short half-life of phentolamine. Different treatments were administered at least 2 days apart. To check for efficacy of blockade, heart rate (f_H) was monitored after subcutaneous injection of the cholinergic agonist pilocarpine hydrochloride (1 mg kg^{-1}, Sigma), the α-adrenergic agonist l-phenylephrine hydrochloride (2 mg kg^{-1}, Sigma) or the β-adrenergic agonist isoproterenol hydrochloride (0.05 mg kg^{-1}, Isuprel, Sterling-Winthrop, Markham, Ontario) at the end of diving sessions after atropine, phentolamine, and propranolol and nadolol injections, respectively. Effects of agonists injected alone, before blockade, were assessed in two animals. Pilocarpine decreased f_H by 40 %, phenylephrine decreased f_H by 60 % and isoproterenol increased f_H by 40 %. In all cases, the agonists had no effect on f_H in muskrats treated with the appropriate antagonist.

Voluntary dives were performed in an indoor 270 cm×122 cm×89 cm tank filled with water to a depth of 60 cm. Water temperature ranged from 8 to 12 ˚C. Muskrats could rest on a 61 cm×43 cm platform located above the water level in one corner of the tank. The tank was divided into four lanes which made an underwater maze when covered with plastic mesh. The animals entered the maze at one end, from the platform, and had to swim to the other end to reach food placed under water. The round trip was 21 m. Muskrats were first left overnight with a low level of water to learn their way around. In the second session, the water level was raised above the maze cover. Data were recorded from the third session. The animal was not given access to the underwater maze for 15 min at the beginning of each session. After drug or saline injection, access to the maze was allowed. There were no observers in the laboratory during free-diving sessions.

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 at the beginning of the session. Drugs were injected and the first forced dive was carried out 5 min later. Dive length was varied randomly from 15 s to 2 min. At least 10 dives were performed within one session. Animals were left alone in the laboratory between dives. The experimenter entered the room about 30 s before each dive and left within the first 15 s after the dive.

The effects of the injected drugs on maximum dive time were estimated in three muskrats. To allow the animals to warm up and to get used to the procedure, they were first left in air for 10 min, then submerged for 2 min, left in air for 10 min, submerged for 4 min and again left in air for 10 min. Maximum dive time was then estimated on the third dive, which was stopped when the animals lost their balance. None of these dives was terminal. Saline, DL-propranolol hydrochloride (4 mg kg^{-1}, Sigma), atropine sulphate (1 mg kg^{-1}, Sigma), phentolamine mesylate (1 mg kg^{-1},

Rogitine, Ciba-Geigy) and a mixture of atropine sulphate (1 mg kg^{-1}) and phentolamine mesylate (1 mg kg^{-1}) were administered in randomized order.

The ECG was monitored during diving sessions using a Konigsberg Instrument telemetry system (Pasadena, California). The ECG signal was recorded on the audio channel of a video recorder after modulation using a Vetter FM recording adaptor (A.R. Vetter Co., Rebersburg, Pennsylvania). The animal's behaviour was recorded on the video channel using a Panasonic camera (Secausus, New Jersey). When tapes were replayed, the ECG signal was demodulated and logged using Labtech Notebook software (Laboratory Technologies Corporation, Wilmington, Massachusetts) 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 times of submersion and surfacing, judged from watching the animal on a television monitor. Subsequently, each inter-beat interval was converted to beats min^{-1}. Mean heart rate over 1 min was calculated during periods of rest (resting f_H) and routine activity such as grooming and eating (routine f_H) in untreated animals. Routine f_H was calculated before and after drug injection in treated muskrats. Mean values from each muskrat (N=5) were averaged to give a grand mean for resting f_H and routine f_H in the untreated group and for routine f_H before and after injection in treated groups. To reduce variability caused by dive duration, only dives lasting more than 40 s and less than 75 s were analysed. The first four dives meeting these criteria were analysed for each animal in each group. Mean f_H during diving (diving f_H) as well as mean f_H during the 15 s preceding a dive (pre-dive f_H) and following a dive (post-dive f_H) were calculated for each dive. 5 s period averages, starting 20 s before dives and ending 20 s after dives, were also computed, to study heart rate profiles during diving. Values from the four dives were averaged in each animal to give a mean for pre-dive f_H, dive f_H, post-dive f_H and every 5 s period. Mean values from each animal (N=5) were then averaged in each group to give a grand mean for each variable. Maximum underwater times from three muskrats were averaged to give a mean for each treatment (N=3).

Values given in the text are the grand mean ± S.E.M. for heart rate and mean ± S.E.M. for maximum dive time. One-way and two-way analyses of variance (ANOVA) 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 calculated with SigmaStat software (Jandel Scientific, San Rafael, California).

Results

Fig. 1 shows the distribution of voluntary dive durations in each group. In all groups, muskrats were able to perform dives for periods as long as or longer than the calculated aerobic dive limit for muskrats (approximately 50 s; MacArthur, 1990).

Fig. 2 shows the effects of diving on mean f_H during voluntary and forced dives in untreated animals. Resting f_H was significantly lower than routine f_H (195±14 beats min^{-1} *versus* 252±13 beats min^{-1} in voluntary dives and 217±11 beats min^{-1} *versus* 262±5 beats min^{-1} in forced dives). Pre-dive f_H (291±9 beats min^{-1}) was significantly higher than routine f_H in voluntary dives and significantly lower (220±12 beats min^{-1}) in forced dives. Diving induced a marked decrease in f_H in both voluntary and forced dives. Mean diving f_H was 112±10 beats min^{-1} in voluntary dives and 62±8 beats min^{-1} in forced dives. The difference in f_H between the two types of dives was significant. In voluntary dives, f_H dropped in 15 s to 104±13 beats min^{-1} and stayed around this value until the last 5 s of the dive, when it started to increase (Fig. 3). In forced dives, f_H at 10 s into the dive was 61±14 beats min^{-1}; it remained at this rate until surfacing (Fig. 3). After surfacing, f_H returned to pre-dive values within the first 5 s in voluntary dives (286±11 beats min^{-1}). Post-dive

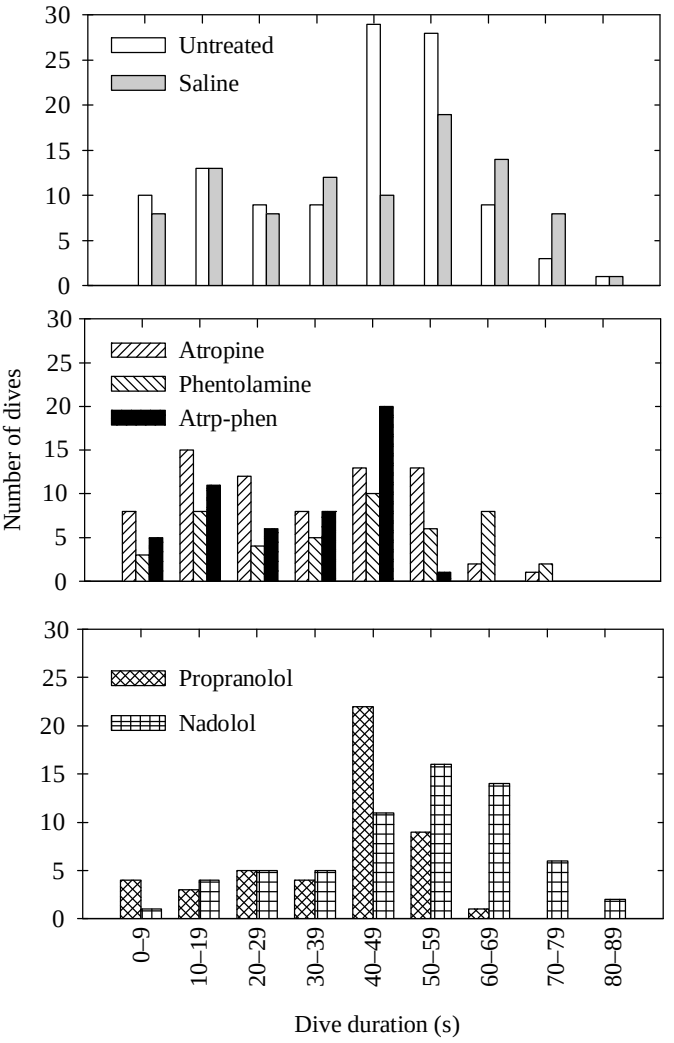

Fig. 1. Distribution of voluntary dive durations in untreated and injected muskrats (N=5). Histograms represent the total number of dives performed by all animals under the various treatment conditions. Atrp-phen; animals injected with atropine and phentolamine.

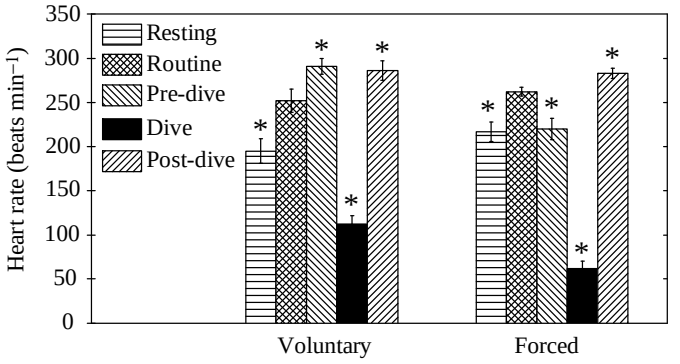

Fig. 2. Mean heart rate (±S.E.M., *N*=5) in untreated muskrats before, during and after voluntary and forced dives. Resting f_H, routine f_H, pre-dive f_H, diving f_H and post-dive f_H are shown. * indicates a value significantly different from routine f_H.

f_H after forced dives (283±6 beats min^{-1}) was not significantly different from post-dive f_H after voluntary dives (Fig. 2).

The effects of injected drugs and diving on mean f_H in the free-diving tank are presented in Fig. 4 and free-diving heart rate profiles are shown in Fig. 5. There was a marked effect of drug treatment on routine f_H. Atropine and phentolamine significantly increased f_H from 244±15 beats min^{-1} to 288±2 beats min^{-1} and from 258±7 beats min^{-1} to 300±12 beats min^{-1}, respectively. Propranolol and nadolol significantly decreased routine f_H from 264±8 beats min^{-1} to 190±12 beats min^{-1} and from 236±10 beats min^{-1} to 183±7 beats min^{-1}, respectively. Saline injection had no effect on routine f_H. Pre-dive f_H did not differ significantly from routine f_H in animals treated with any of the four drugs. Mean diving f_H (304±7 beats min^{-1}) was not significantly different from pre-dive f_H (303±3 beats min^{-1}) in atropine-injected muskrats (Fig. 4) and f_H in these animals remained constant during the dive (Fig. 5). Diving f_H after nadolol (93±12 beats min^{-1}) and phentolamine (117±17 beats min^{-1}) treatment was not significantly different from diving f_H in saline-treated animals (92±10 beats min^{-1}) (Fig. 4), and f_H profiles were similar during diving (Fig. 5). Diving f_H in propranolol-treated animals (138±10 beats min^{-1}) was significantly higher than in saline-treated animals (Fig. 4). f_H at 15 s into the dive was 130±12 beats min^{-1} in this group and it remained around this value until the end of the dive, when it showed the same significant increase as in all the other groups except the atropine-treated group (Fig. 5). f_H returned to predive values within the first 5 s after surfacing in all groups: 296±11 beats min^{-1} in the saline group, 298±6 beats min^{-1} in the atropine group, 307±11 beats min^{-1} in the phentolamine group, 220±6 beats min^{-1} in the nadolol group and 214±12 beats min^{-1} in the propranolol group (Fig. 5).

The effects of injected drugs and submergence on mean f_H in the forced diving cage are presented in Fig. 6 and forced diving heart rate profiles in Fig. 7. Atropine and phentolamine significantly increased routine f_H from 260±12 beats min^{-1} to 287±7 beats min^{-1} and from 247±13 beats min^{-1} to 304±7 beats min^{-1}, respectively. Propranolol and nadolol significantly decreased routine f_H from 249±11 beats min^{-1} to 196±7 beats min^{-1} and from 238±5 beats min^{-1} to 190±5 beats min^{-1}, respectively. Saline injection did not have any effect on routine f_H. Routine f_H in treated animals in the forced diving cage was not significantly different from routine f_H in animals treated with the same drugs in the free-diving tank. Pre-dive f_H dropped significantly compared with routine f_H after drug treatment in every group except for those treated with atropine and propranolol. Nadolol-treated animals had the lowest pre-dive f_H (153±7 beats min^{-1}). Saline- and phentolamine-treated groups had an intermediate pre-dive f_H (228±12 beats min^{-1} and 243±27 beats min^{-1}, respectively). Pre-dive f_H was high in atropine-treated animals (292±6 beats min^{-1}) and low in propranolol-treated animals (181±11 beats min^{-1}). f_H decreased significantly during diving in each group except for that treated with atropine (306±7 beats min^{-1}). Diving f_H in propranolol-treated animals

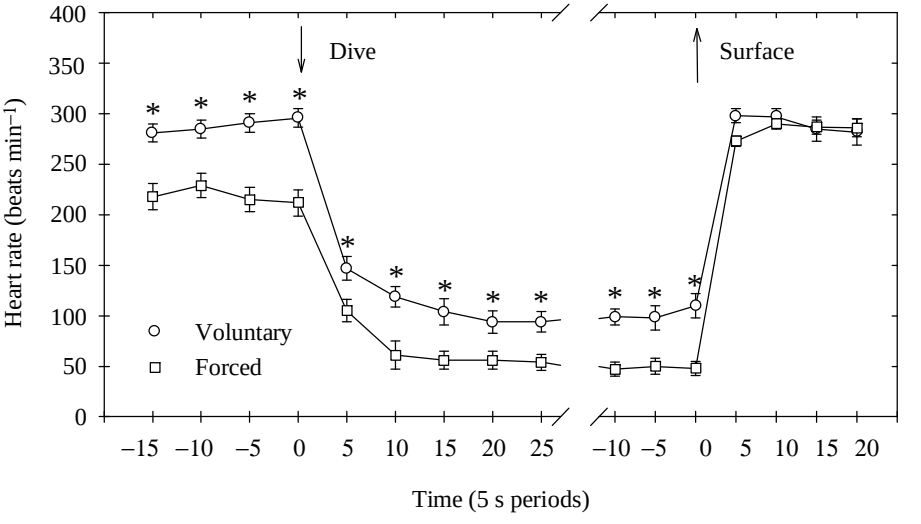

Fig. 3. Heart rate profiles before, during and after voluntary and forced dives in untreated muskrats. In each dive, heart rate was averaged for 5 s before the time values shown and data from all dives were averaged for each animal. Data from all animals (*N*=5) were then averaged to give the means (±S.E.M.) illustrated. The time was set to zero when the animal's nose entered the water and then reset to zero at the end of the dive when the nose broke the surface. The first series of negative time values up to zero represents pre-dive time. The first series of positive time values represents dive time. The second series of negative time values up to zero represents time before the muskrat surfaced. The second series of positive time values represents post-dive time. * indicates values significantly different from heart rate in the corresponding 5 s period in forced dives.

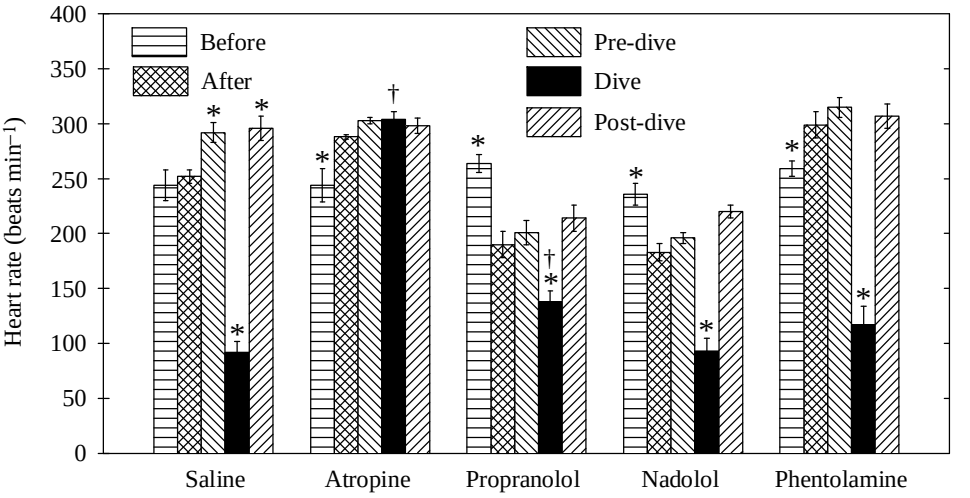

Fig. 4. Effect of injected drugs and submergence on mean heart rate of muskrats (±S.E.M., N=5) in the free-diving tank. Routine f_H before and after drug injection, pre-dive f_H, diving f_H and post-dive f_H are shown for saline-, atropine-, propranolol-, nadolol- and phentolamine-treated muskrats. * indicates values significantly different from routine f_H after drug injection. † indicates that diving f_H differs significantly from diving f_H in saline-treated animals.

(88±9 beats min^{-1}) tended to be higher than in saline- (63±9 beats min^{-1}) and nadolol- (47±7 beats min^{-1}) treated groups but, unlike voluntary dives, differences between these three groups were not significant. Diving f_H in phentolamine-treated animals (79±10 beats min^{-1}) did not differ from that in the saline-treated group. In every group except that treated with atropine, post-dive f_H was significantly higher than pre-dive f_H, but did not differ from routine f_H.

To study the extent to which the diving response enables muskrats to survive under water, we investigated the effects of injected drugs on maximum dive time in forcibly submerged animals (N=3). Maximum underwater endurance was 12.0±1.1 min in untreated muskrats. Maximum dive time was not significantly different from untreated values in the saline (10.6±1.3 min) and propranolol (11.0±0.1 min) groups. Atropine significantly decreased maximum underwater time

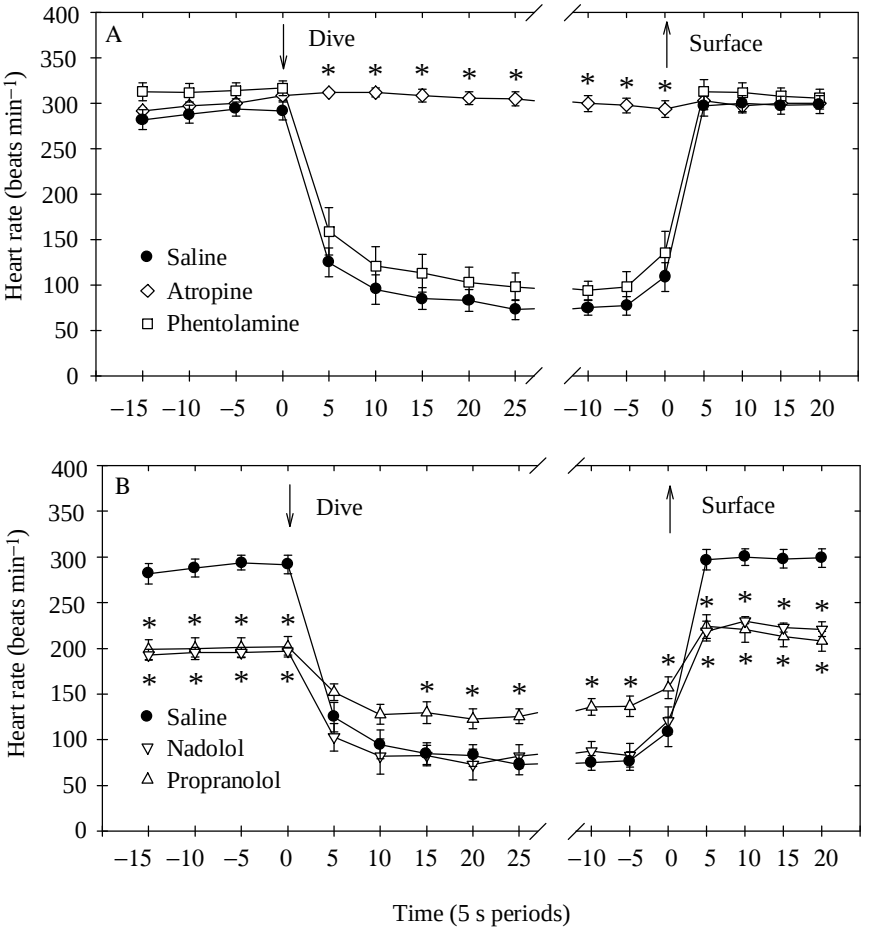

Fig. 5. Heart rate profiles (mean ± S.E.M., N=5) before, during and after voluntary dives in saline-, atropine- and phentolamine-treated muskrats (A) and in saline-, nadolol- and propranolol-treated muskrats (B). Other details as in Fig. 3. * indicates values significantly different from heart rate in the corresponding 5 s period in saline-treated animals.

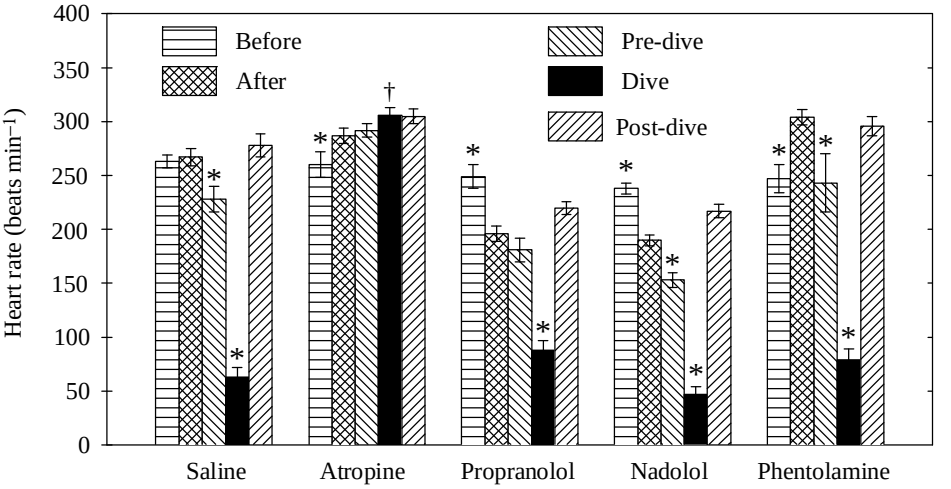

Fig. 6. Effect of injected drugs and submergence on mean heart rate of muskrats (±S.E.M., *N*=5) during unrestrained forced dives (see text). Other details as in Fig. 4.

to 7.7±0.1 min (a 27 % decrease from the saline-treated group) and phentolamine further reduced maximum dive time to 5.2±0.4 min (a 51 % decrease from the saline-treated group). A mixture of atropine and phentolamine had the same effect on maximum dive time (5.2±0.5 min) as phentolamine alone.

Discussion

Diving induced a marked and consistent decrease in f_H in untreated muskrats. f_H dropped to 36 % of pre-dive f_H values or 53 % of resting f_H values in 15 s in free-diving animals, values that compare well with earlier studies in muskrats (Drummond and Jones, 1979; MacArthur and Karpan, 1989; McCulloch and Jones, 1990). Diving bradycardia was consistently present in every dive, no matter how short. In fact, as soon as the muskrat's nose entered the water, f_H slowed down, even if no dive was ultimately performed. This contrasts with responses in seals, which sometimes do not display any bradycardia during short dives (Jones *et al.* 1973), and dabbling ducks, which may not show any reduction in f_H when the head is submerged (Furilla and Jones, 1986*b*). Cardiac deceleration was even more pronounced in forced dives (to 28 % of pre-dive f_H and resting f_H in 10 s). A difference in intensity of bradycardia between voluntary and forced dives has been reported for a number of species (Butler and Woakes, 1979; Furilla and Jones, 1986*a*; Kanwisher *et al.* 1981; Jones *et al.* 1988; MacArthur and Karpan, 1989; McCulloch and Jones, 1990). Intensified bradycardia during forced dives is sometimes explained by the attendant reduction in muscular activity. This was not the case in our experiments, because forcibly submerged muskrats usually swam in a circle or shook the side of the cage. The extreme forced dive bradycardia we observed may result from the presence of an experimenter during this type of dive and/or the absence of control of dive duration by the animals.

The effects of injected drugs on routine f_H were as expected; that is, in agreement with their pharmacological action on the autonomic nervous system (Katzung, 1992). Atropine abolished bradycardia in voluntary and forced dives, confirming the essential role of the parasympathetic system (MacArthur and Karpan, 1989; Murdaugh *et al.* 1961). A surprising result was the reduced free-diving bradycardia in propranolol-treated animals. This effect was not seen with the other β-adrenergic antagonist nadolol. Propranolol is more liposoluble than nadolol and thus crosses the blood–brain barrier more readily (Katzung, 1992). A central effect of propranolol in causing sedation or perhaps blockade of central catecholaminergic pathways may explain the differential effect on diving f_H of the two drugs. Indeed, muskrats treated with propranolol showed a reduced level of activity. They spent most of their time between dives resting on the platform, whereas muskrats in other treatment groups displayed more grooming, eating and exploratory activity. The anaesthetic properties of propranolol, which are not shared by nadolol (Katzung, 1992), could also cause a higher diving f_H by reducing both afferent and efferent peripheral neural activity.

A puzzling result is the absence of any effect of nadolol on diving bradycardia. If diving bradycardia is only due to increased vagal activity and not to sympathetic withdrawal, then blocking cardiac β-adrenergic receptors should further decrease f_H during diving. The explanation that sympathetic tone collapses during diving does not hold because, in that case, atropine-treated animals would have shown a decrease in f_H during submergence. We propose that an accentuated antagonism occurs between the two branches of the autonomic nervous system during diving (Levy, 1971). Intense vagal activity during diving may block sympathetic inputs to the heart, despite the persistence of sympathetic tone, so complete pharmacological sympathetic blockade will not result in a significant decrease of f_H. This means that the parasympathetic system takes over cardiac control during diving and that sympathetic input to the heart is ineffective while the animal is under water. In fact, this would be a very efficient way of rapidly suppressing sympathetic influences because the effective response to changes in sympathetic activation occurs much more slowly than changes due to parasympathetic activity (Akselrod *et al.* 1985; Furilla and Jones, 1987; Japundzic *et al.*

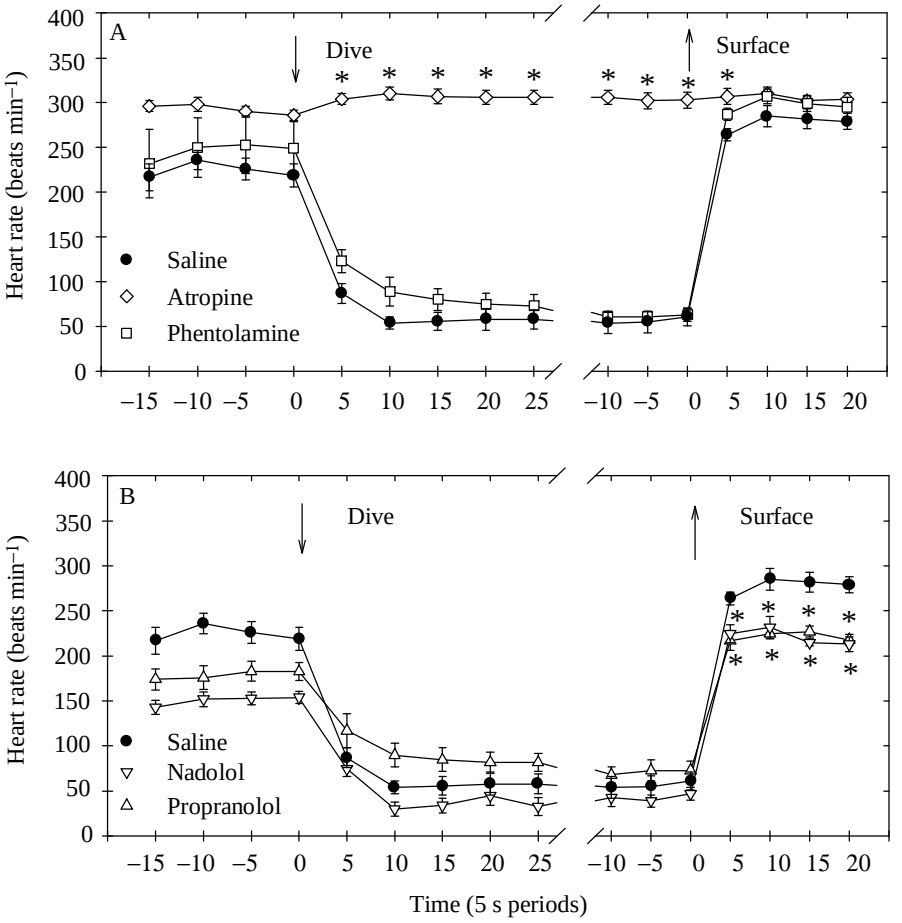

Fig. 7. Heart rate profiles (mean ± S.E.M., *N*=5) before, during and after unrestrained forced dives in saline-, atropine- and phentolamine-treated muskrats (A) and in saline-, nadolol- and propranolol-treated muskrats (B). Other details as in Fig. 3. * indicates values significantly different from heart rate in the corresponding 5 s period in saline-treated animals.

1990). A cholinergically mediated reduction of the response of cardiac cells to adrenergic stimulation during diving may also explain why harbour seals develop a diving bradycardia despite a large increase in circulating epinephrine and norepinephrine levels (Hance *et al.* 1982). Also, mallard ducks forced to dive display the most remarkable increase in circulating catecholamine levels known, yet diving f_H remains low and is unaffected by β-blockade (Butler and Jones, 1971; Lacombe and Jones, 1990). In free-diving ducks, diving f_H increases with sympathetically driven increase in pre-dive f_H. However, diving f_H increases less than pre-dive f_H, suggesting that accentuated antagonism between the two branches of the autonomic nervous system also occurs in voluntarily diving ducks.

Diving bradycardia occurs in association with peripheral vasoconstriction, which is effected by α-adrenergic control (Bron *et al.* 1966; Butler and Jones, 1971; Kobinger and Oda, 1969; Lacombe and Jones, 1991*a*). Some authors have argued that bradycardia in ducks forced to dive is a secondary cardiovascular adjustment to the change in peripheral resistance to maintain blood pressure (Blix *et al.* 1974; Andersen and Blix, 1974). Others have concluded that the baroreflex operates during forced dives but is not responsible for diving bradycardia (Jones *et al.* 1983; Kobinger and Oda, 1969; Smith and Jones, 1992). Our results show that diving bradycardia is unaffected by α-blockade with phentolamine, which extends to free-diving animals the conclusion that diving bradycardia occurs independently from vasoconstriction (Murdaugh *et al.* 1968). Although we could not monitor blood flow during voluntary dives, the increase in f_H after injection of phentolamine together with the lack of effect of the α-adrenergic agonist phenylephrine in phentolamine-treated animals suggest that α-blockade was indeed effective in our animals. Furthermore, phentolamine markedly reduced maximum underwater endurance in forced dives.

The acceleration of f_H before surfacing seen in our muskrats towards the end of voluntary dives has been previously reported in seals (Jones *et al.* 1973; Murdaugh *et al.* 1961; Thompson and Fedak, 1993). It is sometimes explained by a decompression reflex, but this cannot be the case in muskrats because they are shallow divers and swam in 60 cm of water in our experimental set-up. Cardiac acceleration before surfacing was not affected by α- and β-adrenergic blockade and was absent in atropine-injected animals, which suggests that it is caused by withdrawal of vagal inputs. Thus, an early reversal of the diving response seems to take place while the animal is still under water.

Untreated animals showed an increase in f_H from resting to routine conditions which is easily explained by an increase in activity. There was a further increase from routine f_H to pre-dive f_H before voluntary dives. In this case, the level of activity does not appear to be the explanation because muskrats stayed motionless on the edge of the platform for a variable period

ranging from a few seconds to 30 s before diving. Pre-dive tachycardia has been reported to occur simultaneously with hyperventilation in ducks (Butler and Woakes, 1979; Furilla and Jones, 1987), and there is ample evidence for an interaction between respiratory and cardiovascular control systems which would elevate f_H (Daly, 1984). However, in our experiments, the elevation in pre-dive f_H was absent in the propranolol and nadolol groups (no increase was seen in the atropine and phentolamine groups either, but routine f_H was already high in these groups), indicating a strong influence of cardiac sympathetic innervation. Changes in f_H due to lung inflation or increased activity of the inspiratory centres are unaffected by propranolol blockade and predominantly brought about by a reduction in cardiac vagal tone (Akselrod *et al.* 1985; Daly, 1984; Japundzic *et al.* 1990). Therefore, pre-dive tachycardia is of different origin. Pre-dive tachycardia may serve to increase oxygen delivery to the tissues before diving and thus to increase the aerobic capacity of the animal. It is interesting to note that an anticipatory bradycardia has been shown in harbour seals, suggesting a different strategy in preparation for diving in this species (Jones *et al.* 1973). Control muskrats showed a decrease in f_H before forced dives, which was suppressed by atropine but not by nadolol, indicating the role of increased vagal activity. Propranolol-treated muskrats in the forced diving cage did not display as much grooming and exploratory activity as muskrats in other treatment groups. Also, they did not seem to react as strongly when the experimenter entered the room, which may explain why they did not exhibit any pre-dive bradycardia.

During the first seconds after surfacing, free-diving muskrats displayed the same high f_H as just before diving. Post-dive tachycardia correlates with high oxygen consumption during this period (MacArthur and Krause, 1989). It probably reflects repayment of an oxygen debt incurred during diving, intense grooming and perhaps rewarming since body temperature may drop significantly during a dive (MacArthur and Karpan, 1989). Post-dive tachycardia was not seen in nadolol- and propranolol-treated animals, suggesting an increase in sympathetic activity during the recovery period.

Animals treated with atropine and/or phentolamine were still able to make the round trip in the free-diving tank, taking about 50 s, which is as long as their aerobic dive limit (ADL). The ADL was computed from estimated oxygen stores and measurement of post-dive oxygen consumption (MacArthur, 1990). Calculated this way, it represents a theoretical value, defined as the maximum amount of time that an animal can spend under water relying only on aerobic biochemical pathways. The ADL can also be determined empirically by measuring levels of blood lactate, the main metabolite of anaerobiosis. It is then defined as the maximum amount of time spent under water without any significant increase in blood lactate levels. The two values agree well in voluntarily diving Weddell seals (Kooyman *et al.* 1980). Since Weddell seals rarely dive for longer than their ADL in the wild, it has been concluded that this species performs most of its dives aerobically (Kooyman *et al.* 1980). Unfortunately, the ADL value derived from blood lactate measurements is not known for muskrats. However, if muskrats performed their dives primarily aerobically, suppressing the diving response should have significantly decreased the duration of their voluntary dives below their ADL. Since this was not the case in the present experiments, we hypothesize that muskrats may use anaerobic in addition to aerobic biochemical pathways throughout voluntary dives.

Blockade of the diving response with atropine significantly decreased maximum underwater time, and blockade with phentolamine decreased it further. Atropine and phentolamine given at the same time had the same effect as phentolamine alone. This suggests that peripheral vasoconstriction and bradycardia greatly improve underwater endurance in a non-additive manner and that vasoconstriction is more efficient, confirming findings in other species (Andersen and Blix, 1974; Lacombe and Jones, 1991*b*; Murdaugh *et al.* 1968). Nevertheless, muskrats without a cardiac and vasomotor response (atropine–phentolamine group) could stay under water for more than 5 min. This is unusual because harbour seals, for instance, cannot sustain as long a dive in the absence of a diving response (Murdaugh *et al.* 1968). The exceptional underwater endurance of muskrats in the absence of a diving response may be due to the essential role of anaerobic biochemical pathways during diving in this species.

In conclusion, these experiments emphasize the importance of vagal outflow to the heart during voluntary dives and of sympathetic outflow during the pre-dive and post-dive periods. They suggest an accentuated antagonism between the two branches of the autonomic nervous system during diving, so that parasympathetic influences on the heart predominate. Furthermore, they show that free-diving bradycardia may occur independently of peripheral vasoconstriction and that bradycardia and peripheral vasoconstriction greatly improve underwater endurance in muskrats. Finally, this study raises the fundamental question of understanding how muskrats can perform free dives as long as their ADL and survive 5 min forced dives without a diving response.

This research was supported by operating 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) for lending us the ECG telemetry system.

References

AKSELROD, S., GORDON, D., MADWED, J. B., SNIDMAN, N. C., SHANNON, D. C. AND COHEN, R. J. (1985). Hemodynamic regulation: investigation by spectral analysis. *Am. J. Physiol.* **249**, H867–H875.

ANDERSEN, H. T. AND BLIX, A. S. (1974). Pharmacological exposure of components in the autonomic control of the diving reflex. *Acta physiol. scand.* **90**, 381–386.

BERT, P. (1870). *Leçon sur la Physiologie Comparée de la Respiration.* Paris: Baillère. pp. 526–553.

BLIX, A. S., GAUTVIK, E. L. AND REFSUM, H. (1974). Aspects of the relative roles of peripheral vasoconstriction and vagal bradycardia in the establishment of the 'diving reflex' in ducks. *Acta physiol. scand.* **90**, 289–296.

BRON, K. M., MURDAUGH, H. V., MILLEN, J. E., LENTHALL, R., RASKIN, P. AND ROBIN, E. D. (1966). Arterial constrictor response in a diving mammal. *Science* **152**, 540–543.

BUTLER, P. J. AND JONES, D. R. (1971). The effect of variations of heart rate and regional distribution of blood flow on the normal pressor response to diving in ducks. *J. Physiol., Lond.* **214**, 457–479.

BUTLER, P. J. AND WOAKES, A. J. (1979). Changes in heart rate and respiratory frequency during natural behaviour of ducks, with particular reference to diving. *J. exp. Biol.* **79**, 283–300.

DALY, M. DE B. (1984). Breath-hold diving: mechanisms of cardiovascular adjustments in the mammal. In *Recent Advances in Physiology* (ed. P. F. Baker), pp. 201–245. Edinburgh: Churchill Livingstone.

DRUMMOND, P. C. AND JONES, D. R. (1979). The initiation and maintainance of bradycardia in a diving mammal, the muskrat, *Ondatra zibethicus. J. Physiol., Lond.* **290**, 253–271.

DYKES, R. W. (1974). Factors related to the dive reflex in harbor seals: sensory contributions from the trigeminal region. *Can. J. Physiol. Pharmac.* **52**, 259–265.

FURILLA, R. A. AND JONES, D. R. (1986*a*). The contribution of nasal receptors to the cardiac response to diving in restrained and unrestrained redhead ducks (*Aythya americana*). *J. exp. Biol.* **121**, 227–238.

FURILLA, R. A. AND JONES, D. R. (1986*b*). Cardiac responses to dabbling and diving in the mallard, *Anas platyrhynchos*. *Physiol. Zool.* **60**, 406–412.

FURILLA, R. A. AND JONES, D. R. (1987). The relationship between dive and pre-dive heart rates in restrained and free dives by diving ducks. *J. exp. Biol.* **127**, 333–348.

GABBOTT, G. R. J. AND JONES, D. R. (1987). Habituation of the cardiac response to involuntary diving in diving and dabbling ducks. *J. exp. Biol.* **131**, 403–415.

HANCE, A. J., ROBIN, E. D., HALTER, J. B., LEWINSTON, N., ROBIN, D. A., CORNELL, L., CALIGIURI, M. AND THEODORE, J. (1982). Hormonal changes and enforced diving in the harbor seal *Phoca vitulina*. II. Plasma catecholamines. *Am. J. Physiol.* **242**, R528–R532.

JAPUNDZIC, N., GRICHOIS, M.-L., ZITOUN, P., LAUDE, D. AND ELGHOZI, J.-L. (1990). Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J. auton. nerv. Syst.* **30**, 91–100.

JONES, D. R., FISHER, H. D., MCTAGGART, S. AND WEST, N. H. (1973). Heart rate during breath-holding and diving in the unrestrained harbor seal (*Phoca vitulina richardi*). *Can. J. Zool.* **51**, 671–680.

JONES, D. R., FURILLA, R. A., HEIES, M. R. A., GABBOTT, G. R. J. AND SMITH, F. M. (1988). Forced and voluntary diving in ducks: cardiovascular adjustments and their control. *Can. J. Zool.* **66**, 75–83.

JONES, D. R., MILSOM, W. K. AND GABBOTT, G. R. J. (1982). Role of central and peripheral chemoreceptors in diving responses of ducks. *Am. J. Physiol.* **243**, R537–R545.

JONES, D. R., MILSOM, W. K., SMITH, F. M., WEST, N. H. AND BAMFORD, O. S. (1983). Diving responses in ducks after acute barodenervation. *Am. J. Physiol.* **245**, R222–R229.

KANWISHER, J. W., GABRIELSEN, G. AND KANWISHER, N. (1981). Free and forced diving in birds. *Science* **211**, 717–719.

KATZUNG, B. G. (1992). *Basic and Clinical Pharmacology*. Norwalk, USA: Appleton and Lange.

KOBINGER, W. AND ODA, M. (1969). Effects of sympathetic blocking substances on the diving reflex of ducks. *Eur. J. Pharmac.* **7**, 289–295.

KOOYMAN, G. L., WAHRENBROCK, E. A., CASTELLINI, M. A., DAVIS, R. W. AND SINNETT, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. comp. Physiol.* **138**, 335–346.

LACOMBE, A. M. A. AND JONES, D. R. (1990). The source of circulating catecholamines in forced dived ducks. *Gen. comp. Endocr.* **80**, 41–47.

LACOMBE, A. M. A. AND JONES, D. R. (1991*a*). Neural and humoral effects on hindlimb vascular resistance of ducks during forced submergence. *Am. J. Physiol.* **261**, R1579–R1586.

LACOMBE, A. M. A. AND JONES, D. R. (1991*b*). Role of adrenal catecholamines during forced submergence in ducks. *Am. J. Physiol.* **261**, R1364–R1372.

LEVY, M. N. (1971). Sympathetic–parasympathetic interactions in the heart. *Circulation Res.* **29**, 437–445.

MACARTHUR, R. A. (1990). Seasonal changes in the oxygen storage capacity and aerobic dive limits of the muskrat (*Ondatra zibethicus*). *J. comp. Physiol.* B **160**, 593–599.

MACARTHUR, R. A. AND KARPAN, C. M. (1989). Heart rate of muskrats diving under simulated field conditions: persistence of the bradycardia response and factors modifying its expression. *Can. J. Zool.* **67**, 1783–1792.

MACARTHUR, R. A. AND KRAUSE, R. E. (1989). Energy requirements of freely diving muskrats (*Ondatra zibethicus*). *Can. J. Zool.* **67**, 2194–2200.

MCCULLOCH, P. F. AND JONES, D. R. (1990). Cortical influences on diving bradycardia in muskrats (*Ondatra zibethicus*). *Physiol. Zool.* **63**, 1098–1117.

MURDAUGH, H. V., CROSS, C. E., MILLEN, J. E., BERNARD, J., GEE, L. AND ROBIN, E. D. (1968). Dissociation of bradycardia and arterial constriction during diving in the seal *Phoca vitulina. Science* **162**, 364–365.

MURDAUGH, H. V., SEABURY, J. C. AND MITCHELL, W. L. (1961). Electrocardiogram of the diving seal. *Circulation Res.* **9**, 358–361.

RICHET, C. (1894). Influence de l'atropine sur la durée de l'asphyxie chez le canard. *C. R. Hebd. Séanc. Soc. Biol. Paris* **1**, 789–790.

RIDGWAY, S. H., CARDER, D. A. AND CLARK, W. (1975). Conditioned bradycardia in the sea lion, *Zalophus californianus. Nature* **256**, 37–38.

SMITH, F. M. AND JONES, D. R. (1992). Baroreflex control of arterial blood pressure during involuntary diving in ducks (*Anas platyrhynchos* var.). *Am. J. Physiol.* **263**, R693–R702.

THOMPSON, D. AND FEDAK, M. A. (1993). Cardiac responses of grey seals during diving at sea. *J. exp. Biol.* **174**, 139–164.