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EFFECT OF DIAZEPAM ON HEART AND RESPIRATORY RATES OF HARBOR SEAL PUPS FOLLOWING INTRAVENOUS INJECTION

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Capture and immobilization of free-ranging pinnipeds is often required for scientific research, thereby necessitating the use of physical and/or chemical restraint (Gales 1989, Lynch *et al*. 1999). Frequently, when physical restraint (*i.e.,* manual or mechanical) is feasible, a sedative is also administered to increase animal and handler safety, minimize animal distress, and reduce total handling time (Lynch *et al*. 1999, Gulland *et al*. 2001). Diazepam (Valium®, Sternbach and Reeder 1961), a benzodiazepine derivative, has been described as the best ataractic (*i.e.,* sedative or tranquillizer) for phocids (Hubbard 1969). Because of its hypnotic (*i.e.*, sleep-inducing), anxiolytic (*i.e.,* anti-anxiety), anticonvulsant, and muscle relaxant properties (Randall *et al*. 1961), diazepam has been used in several seal species to facilitate a variety of minor procedures from instrumentation and tissue sample collection to force feedings and fiberoptic gastroscopy (*e.g.,* Greenwood and Wild 1977, Gage 1984, Bowen *et al*. 1999). Regardless of the numerous studies using diazepam as a sedative, limited information exists detailing the effects of this drug on the heart and respiratory rates of seals. Research on other mammals, including humans, however, has yielded conflicting results (Rao *et al*. 1972, Kelly *et al*. 1973, Muir *et al*. 1982, Haskins *et al*. 1986, Yang *et al*. 1987, Klein and Reinhold 2001).

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Previous studies on phocid neonates have established that cardiorespiratory control is not fully developed at birth (Castellini *et al*. 1994, Falabella *et al*. 1999, Lapierre *et al*. 2004). Consequently, physiologically immature animals may be more susceptible to experience adverse reactions following drug administration. While studying diving behavior and physiology ontogenesis, harbor seal pups were sedated with diazepam to facilitate blood and muscle sample collection (Greig 2003, Clark 2004). During the course of these studies, we observed pronounced behavioral and physiological changes following injection of diazepam. As a result, the aim of this study was to assess the time-course effects of diazepam on the cardiac and respiratory rates of harbor seal pups during early postnatal development.

This study was conducted near Bic (48°24′ N, 68°51′ W), QC, Canada, on the south shore of the St. Lawrence River Estuary, from May to July of 2002. The effects of diazepam on heart and respiratory rates were evaluated in eighteen preweaned harbor seal pups. Pups ranged in age from 1 d to 33 d and weighed between 9 and 32 kg. Procedures have been described in detail elsewhere (Lapierre 2003, Lapierre *et al*. 2004). Briefly, following tagging and morphometrics, seals were mechanically restrained with a V-board. Subsequently, electrocardiogram (ECG), heart rate (HR), and respiration rate (RR) were simultaneously recorded using a portable multichannel physiological monitor, needle electrodes, laptop computer, and a camcorder.

On average, after 30 min (range 15–52 min) of continuous baseline monitoring (Lapierre *et al*. 2004) animals were sedated with diazepam (Valium®, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one, Sternbach and Reeder 1961) to facilitate tissue sample collection as part of two concurrent studies (Greig 2003, Clark 2004). Each pup was administered a single intravenous (i.v.) bolus injection of diazepam (5 mg mL^{-1}, Sabex Inc., Boucherville, QC, Canada) via the extradural intravertebral vein in the lumbar region (Hubbard 1969). Pups <20 kg and pups ≥ 20 kg were administered 1.0 mL (5 mg) and 1.25 mL (6.25 mg) of diazepam, respectively, resulting in a mean dose of 0.32 ± 0.09 mg kg^{-1}. Blood and muscle biopsy procedures typically lasted 50 min (range 35–64 min). To ensure complete recovery, pups were monitored, on average, for an additional 25 min (range 15–31 min) before removal of electrodes and their subsequent release. Total handing time, from capture to release, lasted on average 2.32 h (range 1.85–4.00 h).

Apnea was observed as a breath-hold, during which nostrils remained closed for >10 s following an exhalation (Lapierre *et al*. 2004). Mean breathing heart rate (HR) (*i.e.,* HR during periods of respiration) and respiration rate (RR) for twelve 5-min epochs following administration of diazepam (t_1 = 0–5 min, t_2 = 5–10 min, t_3 = 10–15 min ... t_{11} = 50–55 min, t_{12} = 55–60 min) were compared to mean baseline measurements (*i.e.,* control, t_0; Lapierre *et al*. 2004). For apnea comparisons, two 30-min epochs (t_1–t_6 and t_7–t_{12}) were identified, and apnea count, mean apnea duration, and mean apnoeic HR were calculated and compared to baseline values (t_0). Induction time was defined as the interval between injection and the appearance of sedation (*i.e.,* calm resting state), and the duration of sedation was defined as the time between induction and the appearance of increased responsiveness.

To determine if diazepam altered HR or RR, all parameters were assessed using a one-way analysis of variance (ANOVA) for repeated measures (PROC GLM, SAS

Institute Inc., Cary, NC). The CONTRAST transformation (SAS Institute Inc. 1989) was selected, whereby one level of the repeated measures effect is considered the control (t_0) against which all other levels are compared. To assess for the effect of age, each pup was assigned to one of the following categories (young = 1–10 d [$n = 7$], mid = 11–20 d [$n = 6$], old = 21–33 d [$n = 5$]). In addition, sex effects were also assessed (n = eight females, ten males). Significance level was set at 0.05. Data are expressed as mean ± standard deviation.

No significant difference by age class or sex was observed for any of the parameters measured; as such, values are reported for all pups combined. Administration of 0.32 ± 0.09 mg kg^{-1} i.v. diazepam provided sufficient sedation to facilitate tissue sample collection. Pups entered a sleep-like state almost immediately; no paradoxical reaction such as excitement or restlessness, as reported in other animals, was observed (Haskins *et al*. 1986, Ko *et al*. 1998). Induction was smooth and rapid, averaging 15 ± 4 s and pups were sedated for 33 ± 13 min, similar to the duration reported for a subadult gray seal administered 0.1 mg kg^{-1} of diazepam (Greenwood and Wild 1977). Prior to release, ~1 h 20 min after administration of diazepam, pups displayed a degree of responsiveness toward handling similar to that observed prior to sedation, suggesting that pups had sufficiently recovered by this time. No fatalities occurred and all seals were re-sighted at a later date appearing in good physical condition.

A significant decline in baseline mean breathing HR ($t_0 = 159 \pm 10$ beats min^{-1}) was observed following administration of diazepam (Fig. 1). The 5-min epochs following drug administration were clustered in three separate groups of statistical significance compared to baseline values and to each other. The most substantial decrease in HR was noted within the first 20 min following diazepam administration (t_1–$t_4 = 137 \pm 28$ beats min^{-1}). The HRs after the first 20 min postadministration, although still significantly different from baseline statistically, were only moderately decreased and not likely clinically significant deviations from baseline (t_5–$t_{11} = 150 \pm 12$ beats min^{-1}). Breathing HR during the last epoch of observation ($t_{12} = 152 \pm 11$ beats min^{-1}) was not statistically different from baseline ($P = 0.0816$). Pups spent a greater proportion of time resting after injection due to the sedative effect of diazepam, and although breathing HR is approximately 10 beats min^{-1} lower during periods of rest (Lapierre 2003), the exaggerated decrease in breathing HR during the first 20 min following diazepam administration was likely the result of increased sympathetic withdrawal (Marty *et al*. 1986), brought about by the anxiolytic properties of diazepam. However, it is likely that an increase in parasympathetic tone was also occurring during this time, as profound bradycardia was observed in two pups. Because no other hemodynamic or cardiovascular parameters were examined, it is difficult to speculate as to which mechanisms contributed to the observed bradycardia. Breathing HR began to stabilize 30–40 min postinjection, remaining approximately 10 beats min^{-1} less than control until the end of the observation period. The greater proportion of time spent resting, rather than a sustained effect of diazepam, likely accounted for this slight, albeit significant, decrease in HR observed during this time.

Increased salivation was noted following injection of diazepam. Atropine (0.02 mg/kg i.m.) has been routinely administered as a premedicant to prevent

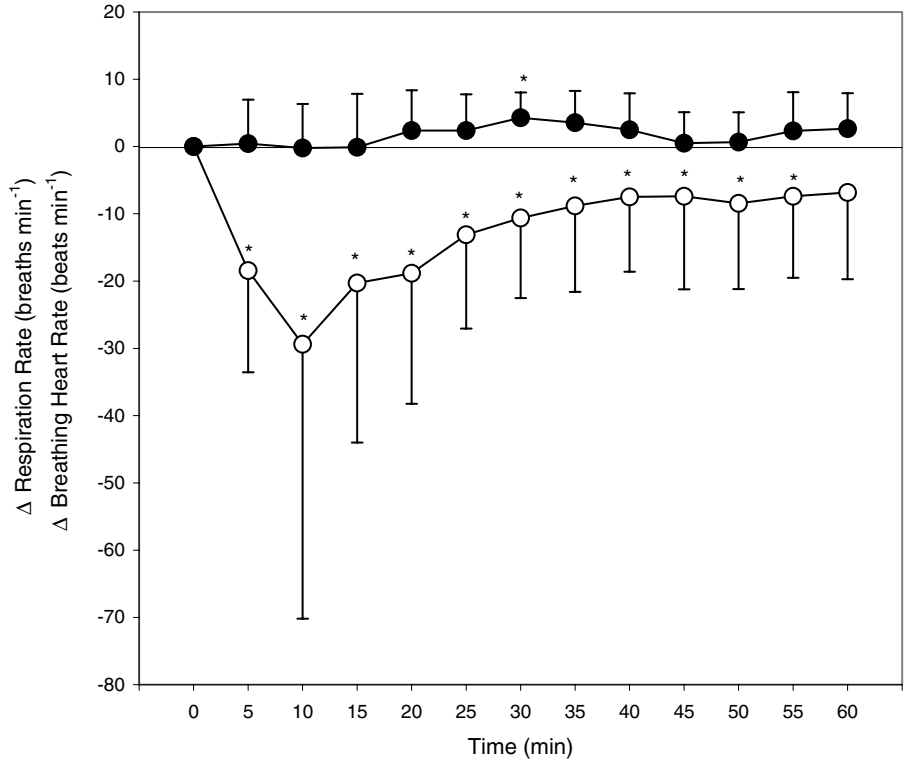

Figure 1. Change in breathing heart rate (○) and respiration rate (●) (mean ± SD) relative to baseline (t_0) following administration of 0.32 ± 0.09 mg kg^{-1} i.v. diazepam for all harbor seal pups combined ($n = 18$). *Statistically different from baseline.

bradycardia and reduce excessive salivation and upper respiratory tract secretion (Haulena and Heath 2001); however, it was not used in this study. Both drug effects (*i.e.,* increased salivation and bradycardia) likely would have been less dramatic had atropine been given. Although, the gold standard of clinical pinniped immobilization and anesthesia includes premedicating with atropine, this practice is not strictly adhered to when working with wild animals in a field setting (*e.g.,* this study, Bowen *et al*. 1999, Baechler *et al*. 2002). In addition, some researchers do not recommend administration of atropine to phocids (Woods, pers. comm. as sited in Haulena and Heath 2001). We suggest that the efficacy of atropine prior to sedation with diazepam in harbor seal pups be further investigated.

Mean breathing frequency was not significantly affected by diazepam; RR for both baseline and postdiazepam epochs (except t_6) was 33 ± 7 breaths min^{-1}, with little variation observed between individuals. The slight significant increase ($P = 0.0407$) in respiration during t_6 (36 ± 6 breaths min^{-1}) was likely a consequence of handling the seal and obtaining the muscle sample, despite the administration of a local anesthetic consisting of a 2% lidocaine block at the biopsy site. Data on

respiration quality could not be obtained from the video footage.

Overall, apnea count (5 ± 3, 5 ± 3, 6 ± 4), mean apnea duration (27 ± 10 s, 33 ± 17 s, 26 ± 18 s), and mean apneic HR (122 ± 15 beats min^{-1}, 102 ± 39 beats min^{-1}, 123 ± 20 beats min^{-1}) for the two 30-min epochs, following administration of diazepam, were not significantly different compared to baseline values (t_0, t_1–t_6, t_7–t_{12}, respectively). However, the majority of pups (fourteen of eighteen), became apneic within 2 min of injection. A transient period of apnea shortly after the administration of an immobilizing or anesthetic agent is a common response in pinnipeds (Gales 1989, Lynch *et al*. 1999, Haulena and Heath 2001). The average duration of this initial apnea, after administration of diazepam (62 ± 51 s), was twice as long as the mean baseline apnea length (27 ± 10 s) ($t_{14} = 2.914$, $P = 0.013$). In addition, this first apnea was the longest apnea recorded during the entire observation period for seven of the pups. The longest initial apnea following delivery of diazepam occurred in a 33-d-old pup and was just over 3 min (183 s); however, most apneas were well below the estimated theoretical aerobic dive limit for harbor seals (~3 min; Bowen *et al*. 1999, Jørgensen *et al*. 2001). Furthermore, these terrestrial apneas were similar in duration to the dives preformed by similarly aged harbor seal pups from the same population (Greaves *et al*. 2005). It is most likely that the observed apneas were physiologically safe; however, in the absence of additional monitoring, this cannot be definitely confirmed. In addition, it is uncertain whether placebo (*i.e.,* saline) or solvent (*i.e.,* diazepam vehicle) injections would have produced a similar response, and as such we cannot conclusively state that the observed apneas were indeed drug induced. The respiratory stimulant doxapram HCl (Dopram®) has been used with varying success in pinnipeds experiencing prolonged immobilization-related apneas (Haulena and Heath 2001); however, it was not required during this study.

Two pups experienced extreme and extended periods of bradycardia following injection of diazepam, for approximately 12.5 and 21 min, respectively. Immediately, both pups displayed a precipitous decline in HR (Fig. 2). In addition, the pups exhibited extended periods of apnea and unusually low breathing frequency during subsequent periods of eupnea, although a normal sinus arrhythmia was maintained. This may have been the result of a pup's individual reaction to a rapid injection. Diazepam is not water soluble and the commercial formulation uses propylene glycol (40%, Sabex Inc.) as a solvent vehicle, which may produce hypotension, bradycardia, apnea, and cardiac arrest upon rapid infusion (Plumb 1995). When used in pediatrics, it is recommended that diazepam be given slowly over a 3-min period at dose of $\leq$0.25 mg kg^{-1} (Roche Pharmaceuticals, Inc. 1997). During this study, however, diazepam was usually delivered within 10 s. Following delivery of diazepam, both pups were heavily sedated and not easily roused; the length of sedation for one pup (27 min) was similar to the mean duration for all pups combined, but the other pup was sedated for an extended period of time (54 min). Diazepam has been reported to produce prolonged CNS depression in neonates due to their inability to metabolize diazepam into inactive end products (Roche Pharmaceuticals, Inc. 1997). Although an animal's response to physical restraint and the subsequent venous puncture are unpredictable, we urge researchers to administer diazepam as slowly as possible to limit the probability of inducing such profound side effects as observed in these two

pups. Flumazenil (Romazicon®), a specific and competitive benzodiazepine receptor antagonist, has been shown to reverse (completely or partially depending on the dose) the effects of benzodiazepines (Roche Pharmaceuticals, Inc. 1997). In pinnipeds, flumazenil has also been administered to reverse sedation induced by two other commonly used benzodiazepines, zolazepam, and midazolam (Karesh *et al*. 1997, Ryon *et al*. 1999, Tahmindjis *et al*. 2003); however, it was not administered to any animal in this study.

Opportunistically, two adult females (57 and 50 kg, respectively) were also captured during this study and monitored for ten 5-min epochs (50 min). Although adult females were subdued following delivery of diazepam (0.35 and 0.40 mg kg^{-1} i.v., respectively), they tended to remain vigilant during most of the observation period. Most pups and both adult females were easily roused if the researcher had difficulty obtaining the serial blood samples or once the muscle biopsy procedure commenced. Typically, adult females maintained a higher level of alertness following these procedures and required an increase in the degree of physical restraint employed, suggesting the sedative effects of diazepam had diminished. Pups, however, generally became docile again within minutes and continued resting until removal of the electrodes and their subsequent release. Breathing HR and RR were not significantly altered following injection of diazepam for these two adult females combined (116 $\pm$ 4 beats min^{-1} and 20 $\pm$ 3 breaths min^{-1} prediazepam administration and 122 $\pm$ 9 beats min^{-1} and 18 $\pm$ 3 breaths min^{-1} postdiazepam administration). In addition, only one of the adult females became apneic after administration of diazepam; the apnea lasted 59 s and was the only apnea observed during the entire sampling period (including during baseline measurements) for these two individuals. It must be noted that, due to the small sample size, the responses of these two individuals are not nec-

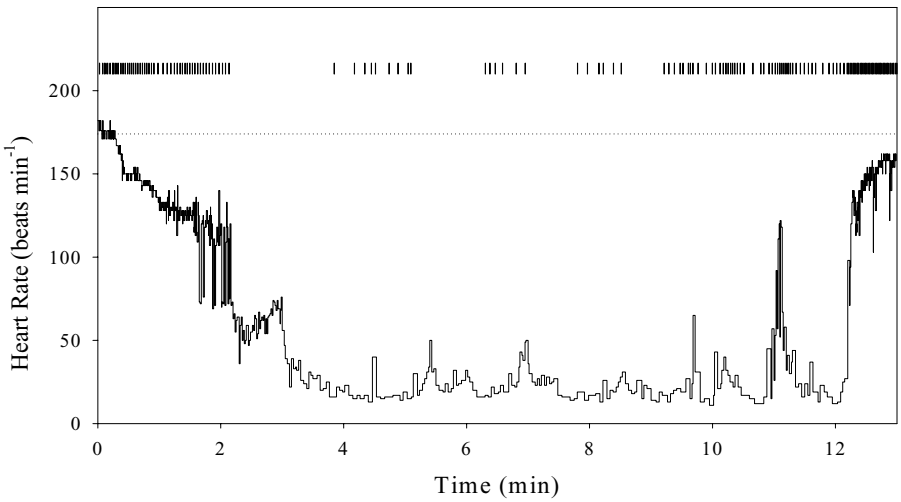

Figure 2. Profound bradycardia following administration of diazepam (0.30 mg kg^{-1} i.v.) for a 19-d-old male pup weighing 21 kg. Baseline heart rate was 174 beats min^{-1} (dotted line). Each vertical tick represents a breath. Similar findings (not shown) were observed for another pup, a 29-d-old male weighing 32 kg and administered 0.23 mg kg^{-1} i.v. diazepam.

essarily representative of the adult seal population in general. Compared to previous studies (0.5–0.7 mg kg^{-1} i.v.; Bowen *et al*. 1999, 2001; Baechler *et al*. 2002), adults in this study were administered a lower dose of diazepam; it is uncertain if a higher dose would have induced physiological changes similar to that observed for pups.

Although diazepam is considered a preferred sedative for phocids, this is the first study, to our knowledge, evaluating the effect of diazepam on heart and respiratory rates when administered as the sole tranquillizer. At a mean dose of 0.32 mg kg^{-1} i.v., diazepam provided sufficient sedation to facilitate blood and muscle collection (Greig 2003, Clark 2004), but pups experienced mild to severe bradycardia following injection. Overall, breathing frequency was unaffected but HR was altered, in some individuals, for upwards of 40 min. Previous studies have achieved successful immobilization with 0.2 mg kg^{-1} (Bowen *et al*. 1999, Baechler *et al*. 2002). It is likely that a lower dose of diazepam and premedicating with atropine would have alleviated some of the undesirable side effects observed in this study. We recommend in future studies that harbor seals be administered a lower dose of diazepam initially (1.5–2.0 mg kg^{-1} i.v., injected as slowly as possible) and subsequently given additional small amounts as deemed necessary. In addition, each animal should be systematically monitored for cardiorespiratory and thermoregulatory disturbances. Furthermore, we suggest that a more detailed study is warranted to further assess the effects of diazepam on the cardiorespiratory parameters of harbor seals and to evaluate the effectiveness of premedicating with atropine in this species.

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