Journal of Zoo and Wildlife Medicine 39(4): 542–547, 2008

DETERMINATION OF A SEDATIVE PROTOCOL FOR USE IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*) WITH NEUROLOGIC ABNORMALITIES UNDERGOING ELECTROENCEPHALOGRAPHIC EXAMINATION

Sophie Dennison, B.V.M.&S., Martin Haulena, D.V.M., M.Sc., D. Colette Williams, B.S., John Dawson, B.S., Brian S. Yandell, Ph.D., and Frances M. D. Gulland, Vet. M.B., Ph.D.

Abstract: Sedation in sea lions exhibiting abnormal neurologic signs may require modification of established sedation protocols because of the likely interaction between effects of the sedative and physiologic changes in diseased animals. The effects of two sedative combinations, 0.07 mg/kg medetomidine and 0.07 mg/kg medetomidine plus 0.2 mg/kg butorphanol, were compared between California sea lions (*Zalophus californianus*) with signs of neurologic dysfunction ($n = 33$) and without neurologic signs ($n = 8$). Sedation depth was scored on a scale of 0 (no effect) to 4 (profound sedation) assessed by response to auditory, tactile, and visual stimuli at the time of perceived maximal sedative effect. In the medetomidine-alone group, sea lions with neurologic signs attained a median sedation score of 4 compared to a median sedation score of 1 in the clinically normal sea lions. Sea lions with and without neurologic signs given medetomidine–butorphanol attained a median sedation score of 4. No statistically significant difference in time to induction and respiratory rate was found between the two sedation protocols in all sea lions. In the sea lions with neurologic signs, the recovery time from medetomidine–butorphanol sedation was prolonged ($P < 0.01$) and minimum recorded heart rates, although remaining within normal physiologic limits, were lower ($P = 0.02$) when compared to the sea lions administered medetomidine alone. Muscle jerks were observed in many animals given medetomidine–butorphanol and were detrimental to the diagnostic quality of the electroencephalogram (EEG) recording. Medetomidine alone at a dose rate of 0.07 mg/kg thus provides adequate and safe sedation in sea lions with neurologic signs undergoing EEG evaluation.

Key words: California sea lion, *Zalophus californianus*, medetomidine, butorphanol, neurologic, electroencephalogram.

INTRODUCTION

Neurologic dysfunction is commonly observed in stranded marine mammals due to a variety of diseases and toxicoses. Encephalitis due to *Toxoplasma gondii*, *Sarcocystis neurona*, morbillivirus, and herpes virus is increasingly reported in cetaceans, sea otters, and pinnipeds.[5,6] Gunshot trauma is responsible for neurologic dysfunction in some animals. Additionally, neurologic signs following natural exposure to domoic acid are common in California sea lions (*Zalophus californianus*) stranding along the west coast of the United States and clinical assessment of these animals is becoming an increasing challenge to the veterinarian.[7] Electroencephalogram (EEG) evaluation is desirable in such sea lions to identify electrical changes associated with clinical neurologic dysfunction.[2,12,13,18]

EEG examination provides an insight into brain function. Data are collected for approximately 25–45 min after placement of electrodes into the subcutum over the cranium.[3] Diagnostic data collection is reliant on minimal muscle movement and muscle tone to optimize EEG quality by reducing the amount of artifact. This may be difficult to achieve in the conscious, free-ranging mammal, necessitating the use of chemical restraint. Anesthesia interferes markedly with brain function, suppresses subclinical electrical activity normally associated with epileptiform patterns on EEG tracings, and induces abnormal electrical complex formation, which affects the diagnostic quality of the EEG examination.[1] As an alternative, profound sedation with α-2 agonists effectively induces a state similar to natural sleep without adversely effecting the EEG data.[25] Interestingly, epileptic episodes in humans have been associated with the onset of sleep, sug-

From The Marine Mammal Center, 1065 Fort Cronkhite, Sausalito, California 94965, USA (Dennison, Gulland, Haulena); Departments of Statistics, and Biostatistics and Medical Informatics, University of Wisconsin, Madison, Wisconsin 53706, USA (Dawson); Departments of Statistics and Horticulture, University of Wisconsin, Madison, Wisconsin 53706, USA (Yandell); and School of Veterinary Medicine, University of California, One Shields Avenue, Davis, California 95616, USA (Williams). Present addresses: (Dennison) Department of Radiology, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, Wisconsin 53706, USA; (Haulena) Vancouver Aquarium, 845 Avison Way, Vancouver, British Columbia V6G 3E2, Canada. Correspondence should be directed to Dr. Dennison (dennison@svm.vetmed.wisc.edu).

gesting that if subclinical epileptiform patterns are present, they may be more easily identified by induction of a sleep state.[12,13,18,22]

Sedation may be complicated by preexisting medical conditions including those resulting in neurologic dysfunction.[7,10] Among sedative and anesthesia protocols described for pinnipeds, medetomidine and butorphanol are commonly used either alone or in combination. Medetomidine, an α-2 agonist, produces sedation and analgesia in a dose-dependent manner with common doses for pinnipeds varying from 0.01–0.14 mg/kg.[8–10,23] Medetomidine has the additional benefit of being completely reversible by the administration of atipamezole.[4,24] Butorphanol, an agonist–antagonist opioid, produces sedative and analgesic effects via σ and κ receptors. It has a synergistic effect when used with medetomidine and has been used in combination with α-2 agonists to produce better relaxation and a longer duration of sedation without increasing adverse side effects in many domestic species and in pinnipeds.[8–11,14–17,23] The aim of this study was to determine a safe, effective sedative regime to facilitate EEG data collection in neurologic California sea lions.

MATERIALS AND METHODS

During May–July 2005, 33 California sea lions stranded along the central California coast with neurologic abnormalities and were admitted to The Marine Mammal Center, Sausalito, California, for treatment. Abnormal neurologic signs, from hereon termed ''neurologic,'' included abnormal pacing, flipper-chewing, head-bobbing and -weaving, periods of absence or loss of awareness of surroundings, seizures, unpredictable or misplaced aggression, transient blindness, coma, muscle fasciculation, and ataxia.

Each sea lion underwent sedation to facilitate diagnostic procedures including blood sampling and EEG. In addition to these 33 sea lions, eight sea lions admitted to The Marine Mammal Center for other reasons but considered neurologically normal required sedation for further diagnostic procedures.[6] Sedation group assignment was achieved by alternating drug protocols between animals. For the purposes of this publication, the sea lions without signs of neurologic abnormalities will, from hereon, be referred to as ''normal.''

Twenty-nine sea lions (five normal and three neurologic males; 21 neurologic females) with a mean body weight of 40 kg (range 20–60 kg) were sedated with 0.07 mg/kg medetomidine (Domitor®, Orion Pharma, Orion Corporation, Espoo, FI-02101, Finland) administered by i.m. injection using a 20G 1 1/2-inch needle into the muscles overlying the tibia and fibula.[10] Twelve sea lions (three normal and three neurologic males; six neurologic females) with a mean weight of 49 kg (range 28–70 kg) were sedated with 0.07 mg/kg medetomidine combined in the same syringe with 0.2 mg/kg butorphanol (Butorject®, Phoenix Pharmaceutical Inc., St. Joseph, Missouri 64503, USA) administered i.m. as described previously.

Table 1. Sedation score, clinical assessment of the level of sedation, and response to visual, auditory, and tactile stimuli in the California sea lions at the time of maximum sedation.

Sedation score	Sedation level	Response to stimuli
0	No effect	Responds normally to auditory and visual stimuli. Normal ambulation or minimal ataxia. Too alert to allow tactile stimulation.
1	Mild sedation	Able to lift head and moves location in response to visual or auditory stimuli. Mild ataxia. Too alert to allow tactile stimulation.
2	Moderate sedation	No response to visual stimuli. May respond to loud auditory stimuli and does respond to tactile stimuli by moving head and/or body. Unable to move location.
3	Good sedation	No response to visual or auditory stimuli. Minimally responds to tactile stimuli by moving eyes but not head or flippers. No attempt to move location.
4	Profound sedation	No response to visual, auditory or tactile stimuli. Snoring may be heard.

Time to maximum effect was recorded as the time post-injection after which there was no further progression of the depth of sedation. The sea lion's response to tactile stimulation was tested by the placement of a 16G 1 1/2-inch needle subcutaneously between the dorsal edges of the scapulae to administer fluids. Sedation was graded on a scale of 0–4 as described in Table 1. Heart rate and respiratory rate were recorded at the time of maximal sedative effect (T = 0) and 15 and 30 min later (T = 15 and T = 30, respectively).

Following EEG of 25–40 min duration, all animals underwent full anesthetic induction using 5% isoflurane (Isoflo®, Abbott Animal Health, Abbott Park, Illinois 60064, USA) administered by mask.[10]

General anesthesia lasted between 25–30 min with 1–1 1/2% isoflurane sufficient to maintain a surgical plane, and facilitated procedures appropriate to the individual animal including radiography, other diagnostics, and wound debridement. When isoflurane administration ceased, all sea lions were administered 0.25 mg/kg atipamezole (Antisedan®. Orion Pharma, Orion Corporation, Espoo, FI-02101, Finland) i.m. as described above to reverse the effects of medetomidine. This dose of atipamezole has been previously described in this species.[8–10] Time from administration of the reversal agent to recovery, defined as the time the animal was able to sit, was recorded and compared. The endotracheal tube was left in place until the animal was able to remove it by coughing. All animals were monitored throughout all procedures continuously and parameters were recorded at 5-min intervals. Where possible, end-tidal carbon dioxide ($EtCO_2$), oxygen saturation (SpO_2), heart rate, respiratory rate, and rectal temperature were all recorded.

Because of the severity of real-time EEG findings, five neurologic animals were euthanized prior to recovery (Goldstein, pers. comm.). Euthanasia was achieved by administering 1 ml/5 kg of 39% pentobarbital sodium and 5% phenytoin sodium (Beuthanasia-D special®, Schering-Plough Animal Health Corp., Union, New Jersey 07083, USA) into the subclavian vein. Data collected from these animals up to the point of euthanasia were included in the statistical analysis.

Comparisons of discrete variables between groups, such as sedation level or numbers of euthanized animals, were examined using Fisher's exact test. Comparisons involving continuous variables, such as heart or respiratory rates, were examined using Welch's *t*-test (Welch–Satterthwaite *t*-test). Statistical analyses were carried out using R (The R Foundation for Statistical Computing, % Department of Statistics and Mathematics, Vienna University of Technology, Karsplatz 13, 1040 Vienna, Austria).

RESULTS

All animals administered the medetomidine–butorphanol combination, and all of the neurologic animals administered medetomidine alone reached a level of sedation sufficient to achieve a diagnostic EEG (sedation score 3 or 4). Medetomidine alone did not provide adequate sedation in any of the normal animals ($n = 5$) (Table 2).

At the nominal time of maximal effect, heart rates in the medetomidine–butorphanol group were significantly lower than those in the medetomidine group (Welch's *t*-test, $P < 0.01$). This result retained its significance when the comparison was restricted to only the neurologic animals (Welch's *t*-test, $P = 0.03$). However, an examination of the data revealed that the lowest heart rates were often recorded at 15 or 30 min post–maximal effect. In light of this, the lowest recorded heart rates were also compared. Minimal heart rates in the medetomidine–butorphanol group were significantly lower than those in the medetomidine group (Welch's *t*-test, $P < 0.01$). Once again, significance withstood restriction to only the neurologic animals (Welch's *t*-test, $P = 0.02$). There was no significant difference in respiratory rate between any two subgroups or overall with respect to sedation regimen (Welch's *t*-test, all $P > 0.7$).

Interestingly, the time to recovery was significantly longer for the neurologic animals given medetomidine–butorphanol compared to those given medetomidine alone (Welch's *t*-test, $P < 0.0001$). However, the caveat with this analysis is that the neurologic medetomidine–butorphanol subgroup had a very low sample size as a smaller number of animals were given this combination and four of the five euthanized animals belonged to this subgroup. When normal animals were included in the calculations, the difference in recovery times between the medetomidine–butorphanol and medetomidine-alone groups was no longer statistically significant (Welch's *t*-test, $P = 0.18$). There was no significant difference between the groups when considering isoflurane concentration or duration of anesthesia ($P = 0.2$). There was no significant difference between any of the groups for the time to reach maximal sedative effect (Welch's *t*-test, all $P > 0.05$). Importantly, there was a difference between the sedative level achieved when the sedative regimens were compared within subgroups. All neurologic animals achieved adequate sedation, regardless of the sedation regimen given. In contrast to the neurologic sea lions, the normal sea lions administered medetomidine–butorphanol reached an adequate level of sedation in two out of three instances, whereas adequate sedation was never achieved for any of the five normal animals given medetomidine alone. Although suggestive, these results were not statistically significant (Fisher's exact test, $P = 0.1$). All $EtCO_2$, SpO_2, and rectal temperature results were within normal limits for all animals and so were not subjected to statistical analysis.[10]

A total of 12 animals were assigned to the medetomidine–butorphanol group before this protocol was terminated. Discontinuation of the protocol was necessary because of excessive muscular jerks

Table 2. Summary of parameters measured following sedation administration. T = time the parameter was measured where T = 0 represents the time of maximal sedative effect and T = 15 and T = 30 are 15 and 30 min later than T = 0, respectively. Heart rate values are separated out because of significant differences identified during statistical analysis at different times. No such differences were identified with the other parameters measured so the data have been grouped all together.

Sedation used and health status	Heart rate, beats per minute. Range (mean ± SD)	Respiratory rate, beats per minute. Range (mean ± SD)	Time to maximum sedative effect, minutes. Range (mean ± SD)	Median sedation score	Time to recovery, minutes. Range (mean ± SD)
Medetomidine					
Neurologic (n = 24)	T = 0: 30–70 (47 ± 12) T = 15: 26–68 (43 ± 13) T = 30: 29–62 (50 ± 13)	6–12 (8 ± 13)	6–15 (9 ± 3)	4	5–46 (16 ± 11)
Normal (n = 5)	T = 0: 32–76 (51 ± 17) T = 15: 24–52 (38 ± 20) T = 30: 48 (48 ± 0)	6–9 (8 ± 2)	3–10 (8 ± 4)	1	3–39 (12 ± 15)
Medetomidine and butorphanol					
Neurologic (n = 9)	T = 0: 28–48 (37 ± 8) T = 15: 28–42 (36 ± 6) T = 30: 30–60 (42 ± 11)	4–11 (9 ± 6)	7–15 (9 ± 3)	4	20–34 (16 ± 4)
Normal (n = 3)	T = 0: 28–44 (33 ± 11) T = 15: 36–42 (38 ± 3) T = 30: 36–48 (44 ± 8)	7–8 (8 ± 1)	7–20 (13 ± 7)	4	2–20 (9 ± 10)

and motion causing marked artifact on the EEG recordings and resulting in nondiagnostic data. Similar motion was not experienced in the medetomidine-alone group.

A total of five animals were euthanized while under sedation because of the severity of the real-time EEG findings. Four of the five were from the medetomidine–butorphanol group. When the final outcome for the animals included in the study was examined, euthanasia was not associated with sedative group allocation as might be suggested if outcome during sedation was considered in isolation.

DISCUSSION

Both sedation protocols provided safe sedation in the neurologic sea lions. Safe sedation was defined as vital parameters remaining within normal physiologic ranges during the period of the sedation and no deaths attributable to the sedation. Only medetomidine–butorphanol provided adequate sedation in the normal animals indicating that the enhanced sedation effect of the medetomidine was related to the neurologic dysfunction. The observation of involuntary myoclonic muscle jerks in the sea lions given medetomidine–butorphanol in combination was not expected. In the canine literature, butorphanol in combination with other pharmaceuticals is cited as abolishing myoclonic jerks induced by medetomidine use alone.[4,11,14–17,19]

Bradycardia, bradypnea, and apnea have been previously described in domestic species and pinnipeds when medetomidine was used alone or in combination with other pharmaceuticals.[4,8–11,14–17,19,23] Alpha-2 agonists typically induce bradycardia, a reduction in cardiac output and peripheral hypotension. However at sedative doses, hypertension may be seen due to peripheral vasoconstriction and a secondary increase in cardiac afterload. Atropine, an anticholinergic pharmaceutical, has been used in pinniped sedation protocols in an attempt to minimize bradycardia induced by a vagal response.[8–10] However, anticholinergic pharmaceuticals are chronotropic and the resultant increased heart rate potentiates hypertension and increases myocardial oxygen tension, demand, and workload. A study of the effects of atropine in dogs administered medetomidine concluded that severe cardiovascular changes should preferentially be addressed by the administration of atipamezole to directly antagonize medetomidine.[21] Relative bradycardia was identified in all the sea lions used for this study, but was most notable within the neurologic animals at 15 min after medetomidine–butorphanol injection. No heart rate ever dropped beneath physiologically normal rates.[10,20] Thus neither reversal nor the administration of atropine was deemed necessary in this study.

The delayed minimal heart rate likely reflects that the time defined as maximal sedative effect was underestimated by the method of assessment. Instead, time to maximal sedative effect should be considered as time to profound sedation in this study. EEG would be an ideal method for monitoring maximal sedative effect with respect to brain function. With careful timekeeping and retrospective assessment of the EEG recording, the exact time to maximal sedative effect may be determined.

The increased likelihood of an animal being euthanized prior to recovery after medetomidine–butorphanol administration may be attributed to a couple of factors: 1) neurologic status was more severe in these animals prior to sedation despite efforts for randomization and 2) butorphanol administration elicited more subclinical epileptiform activity allowing detection of more subtle lesions and a resultant poorer prognosis. Interestingly, when the final long-term outcome for neurologic sea lions from both sedative groups was considered, the percentage of animals within each group that were euthanized equalized. None of the normal animals were euthanized.

The low numbers of normal animals recruited for this study is a study design limitation but the aim of the study was to determine the sedative protocol best suited to the neurologic animals. The ''normal'' data were therefore included purely for the purposes of subjective comparison rather than to examine sedative protocols in the normal animals themselves. Regardless, the data do demonstrate that in the five normal sea lions, medetomidine alone did not provide sufficient sedation for safe handling. The authors recommend the additional use of butorphanol when sedating clinically normal California sea lions.

Given the complete reversibility, profound sedation, faster recovery time, and decreased financial cost when using medetomidine alone compared to medetomidine–butorphanol in combination, the authors recommend medetomidine at a dose rate of 0.07 mg/kg i.m. for use in neurologic California sea lions undergoing EEG examination.

Acknowledgments: The authors would like to thank Michelle Blascow, Amber Makie, and Deb Wickham for their assistance with sedation and treatment of the sea lions, and Shirley Sichel for funding the veterinary internship at The Marine Mammal Center.

LITERATURE CITED

1. Akrawi, W. P., J. C. Drummond, C. J. Kalkman, and P. M. Patel. 1996. Comparison of the electrophysiologic characteristics of EEG burst suppression as produced by isoflurane, thiopental, etomidate and propofol. J. Neurosurg. Anesthesiol. 8: 40–46.

2. Bazil, C. W., and T. S. Walczak. 1997. Effects of sleep stage on epilepsy and non-epileptic seizures. Epilepsia 38: 56–62.

3. Bergamasco, L., A. Accatino, L. Priano, G. Neiger-Aeschbacher, S. Cizinauskas, and A. Jaggy. 2003. Quantitative electroencephalographic findings in beagles anesthetized with propofol. Vet. J. 166: 58–66.

4. Clarke, K. W., and G. C. W. England. 1989. Medetomidine, a new sedative-analgesic for use in the dog and its reversal with atipamezole. J. Small Anim. Pract. 30: 343–348.

5. Dubey, J. P., R. Zarnke, N. J. Thomas, S. K. Wong, W. Van Bonn, M. Briggs, J. W. Davis, R. Ewing, M. Menske, O. C. H. Kwok, S. Romand, and P Thulliez. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona* and *Sarcocystis canis*–like infections in marine mammals. Vet. Parasitol. 116: 275–296.

6. Greig, D. J., F. M. D. Gulland, and C. Krueder. 2005. A decade of live California sea lion (*Zalophus californianus*) strandings along the central California coast: causes and trends 1991–2000. Aquat. Mamm. 31: 11–22.

7. Gulland, F. M., M. Haulena, D. Fauquier, M. F. Lander, T. Zabka, R. Duerr, and G. Langlois. 2002. Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. Vet. Rec. 150: 475–480.

8. Haulena, M., and F. M. D. Gulland. 2001. Use of medetomidine–zolazepam–tiletamine with and without atipamezole reversal to immobilize captive California sea lions. J. Wild. Dis. 37: 566–573.

9. Haulena, M., F. M. D. Gulland, D. G. Calkins, and T. R. Spraker. 2000. Immobilization of California sea lions using medetomidine plus ketamine with and without isoflurane and reversal with atipamezole. J. Wild. Dis. 38: 124–130.

10. Haulena, M., and R. B. Heath. 2001. Marine mammal anesthesia. *In:* Dierauf, L. A., and F. M. D. Gulland (eds.). CRC Handbook of Marine Mammal Medicine, 2nd ed. CRC Press, Boca Raton, Florida. Pp. 655–670.

11. Hayashi, K., R. Nishimura, A. Yamaki, H. Kim, S. Matsunaga, N. Sasaki, and A. Takeuchi. 1994. Comparison of the sedative effects induced by medetomidine, medetomidine–midazolam, and medetomidine–butorphanol in dogs. J. Vet. Med. Sci. 56: 951–956.

12. Janz, D. 1962. The grand mal epilepsies and the sleeping–waking cycle. Epilepsia 3: 69–109.

13. Kellaway, P. 1985. Sleep and epilepsy. Epilepsia 26(Supp. 1): 15–20.

14. Ko, J. C., J. E. Bailey, L. S. Pablo, and T. G. Heaton-Jones. 1996. Comparison of sedative and cardiorespiratory effects of medetomidine and medetomidine–butorphanol combination in dogs. Am. J. Vet. Res. 57: 535–540.

15. Ko, J. C., S. M. Fox, and R. F. Mandsager. 2000. Sedative and cardiorespiratory effects of medetomidine, medetomidine–butorphanol, and medetomidine–ketamine in dogs. J. Am. Vet. Med. Assoc. 216: 1578–1583.

16. Kuo, W. C., and R. D. Keegan. 2004. Comparative cardiovascular, analgesic, and sedative effects of medetomidine, medetomidine–hydromorphone, and medetomidine–butorphanol in dogs. Am. J. Vet. Res. 65: 931–937.

17. Lerche, P., and W. W. Muir. 2004. Effect of medetomidine on breathing and inspiratory pattern drive in conscious dogs. Am. J. Vet. Res. 65: 720–724.

18. Mendez, M., and R. A. Radthke. 2001. Interactions between sleep and epilepsy. J. Clin. Neurophysiol. 18: 106–127.

19. Muir, W. W., J. L. Ford, G. E. Karpa, E. E. Harison, and J. E. Gadawski. 1999. Effects of intramuscular administration of low doses of medetomidine and medetomidine–butorphanol in middle-aged and old dogs. J. Am. Vet. Med. Assoc. 215: 1116–1120.

20. Ponganis, P. J., G. L. Kooyman, L. M. Winter, and L. N. Starke. 1997. Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 167: 9–16.

21. Short, C. E. 1991. Effects of anti-cholinergic treatment on the cardiac and respiratory systems in dogs sedated with medetomidine. Vet. Rec. 129: 310–313.

22. Shouse, M. N., A. M. Da Silva, and M. Sammaritano. 1996. Circadian rhythm, sleep, and epilepsy. J. Clin. Neurophysiol. 13: 32–50.

23. Spelman, L. H. 2004. Reversible anesthesia in captive California sea lions (*Zalophus californianus*) with medetomidine, midazolam, butorphanol and isoflurane. J. Zoo Wildl. Med. 35: 65–69.

24. Vainio, O., and T. Vaha-Vahe. 1990. Reversal of medetomidine sedation by atipamezole in dogs. J. Vet. Pharmacol. Ther. 13: 15–22.

25. Williams, D. C., M. R. Aleman, T. A. Holliday, D. J. Fletcher, R. A. Fletcher, R. A. LeCouteur, and E. P. Steffey. 2003. Sedative effects and stages of arousal and sleep in the normal equine electroencephalogram [CD]. 8th World Congress of Veterinary Anesthesia Conference Proceedings.

Received for publication 30 June 2007