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## IMMOBILIZATION OF JUAN FERNANDEZ FUR SEALS, *ARCTOCEPHALUS PHILIPPII*, WITH KETAMINE HYDROCHLORIDE AND DIAZEPAM

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**ABSTRACT:** During November and December of 1988, 1990, and 1991, a total of 22 free-ranging Juan Fernández fur seal (*Arctocephalus philippii*) females from Alejandro Selkirk Island, Juan Fernández Archipelago, Chile, were immobilized with a combination of ketamine and diazepam. Atropine sulphate was used to decrease respiratory secretions. The mean ( $\pm$ SD) induction dosages of ketamine and diazepam were  $3.64 \pm 1.3$  mg/kg and  $0.12 \pm 0.07$  mg/kg, respectively. Mean ( $\pm$  SD) induction time and time to recovery for females injected intramuscularly (IM) ( $15 \pm 7$  min and  $47 \pm 16$  min) were significantly greater than for females injected intravenously (IV) ( $0.6 \pm 0.4$  min and  $26 \pm 11$  min). Mean ( $\pm$  SD) heart rates and core temperatures were significantly higher for females injected IV ( $173 \pm 15.71$  beats/min and  $37.6 \pm 0.83$  C) than for females injected IM ( $135 \pm 27.06$  beats/min and  $36.5 \pm 1.15$  C). In addition, the IV route resulted in better levels of immobilization compared to the IM route. The degree of immobilization was not related to the dosages of ketamine and diazepam administered. Two animals died after drug administration.

**Key words:** Immobilization, ketamine HCl, diazepam, Juan Fernández fur seal, *Arctocephalus philippii*.

### INTRODUCTION

Field studies that involve the manipulation of free-ranging pinnipeds have been limited by the difficulty and danger of handling these animals (Geraci, 1973), they require the use of physical and chemical restraint techniques to conduct clinical examinations and collect biological samples. Some limitations on physical restraint used alone are related to the weight and strength of the animal and to the amount of stress this technique causes (Gales, 1989). Under such circumstances, it is preferable to use physical restraint in conjunction with immobilizing or anesthetic drugs.

Pinnipeds can be chemically immobilized with a wide range of drugs. Ketamine hydrochloride (HCl), in conjunction with diazepam or xylazine hydrochloride, has been used successfully in several species of phocids and otariids (Gales, 1989; Shaughnessy, 1991). However, the only report on the chemical restraint of Juan Fernández fur seals (*Arctocephalus philippii*) is that of Cardenas and Cattán (1986), who evaluated the use of xylazine in three adults, two juveniles, and one pup.

As part of a long-term study on the ecology, behavior, and physiology of the Juan Fernández fur seal, we evaluated the use of ketamine HCl in conjunction with diazepam as immobilizing drugs for the collection of milk, the deployment of telemetric instruments, and the conduction of stomach lavages in females. Our objectives were to determine the induction time, recovery time, maximum degree of immobilization, and effects on heart rate, respiratory rate, and core temperature of immobilization with ketamine and diazepam.

### MATERIALS AND METHODS

Field work was conducted at Alejandro Selkirk Island, Juan Fernández Archipelago, Chile ( $33^{\circ}45'S$ ;  $80^{\circ}45'W$ ), during the Juan Fernández fur seal breeding seasons (November and December) of 1988, 1990, and 1991. A total of 22 adult, early lactating females weighing a mean  $\pm$  SD of  $46 \pm 8$  kg (range, 35 to 61 kg) were chemically immobilized for tagging, milking, and intubation for collection of stomach contents. Each animal was captured with a circular net and physically restrained in a wooden restraint board (Gentry and Holt, 1982) located approximately 40 to 60 m from the site of capture. Once restrained, the animals were injected

and then weighed. Drug dosages were calculated after administration.

Drugs used were ketamine HCl (Ketostop®, 100 mg/ml, Drag Pharma, Santiago, Chile), and diazepam (Diazepam, 5 mg/ml, Laboratorio Chile, Santiago, Chile) in dosages ranging from 2.16 to 6.76 mg/kg and 0.04 to 0.28 mg/kg, respectively. Atropine sulphate (Atropina Sulfato, 1 mg/ml, Laboratorio Chile) was used in seven animals to decrease respiratory secretions in dosages ranging from 0.002 to 0.005 mg/kg. In addition, doxapram hydrochloride (Viviram®, 100 mg/ml, Drag Pharma) was administered in one female with a marked apnea.

Drugs were injected simultaneously either intramuscularly (IM,  $n = 12$ ) or intravenously (IV,  $n = 10$ ) using 5 ml disposable syringes and 18 gauge 1½ inch needles. Intravenous injections were made into either the extradural veins at the level of the front flippers, or the cephalic vein on the ventral surface of the front flipper. Intramuscular injections were made into the dorsal gluteal muscles, where the blubber layer is thinnest (Trillmich and Weisner, 1979).

To avoid hyperthermia, females were captured either in the early morning or in the late evening, and their flippers were kept wet constantly. Core temperatures were monitored with a digital probe rectal thermometer, and respiratory and heart rates were determined by observation of thoracic movements and palpation at the left fourth intercostal space, respectively. These variables were recorded every 5 min throughout the complete immobilization. In addition, heart and respiratory rates of 10 resting non-immobilized females were determined by observation with binoculars at a distance of 2 to 3 m; heart rates were evident on the left side of the thoracic cage, especially when the fur was wet.

Induction time was defined as the interval between injection and appearance of immobilization and superficial anesthesia. Time to recovery was defined as the interval between the first appearance of drug effects to the time when the animals could not be handled easily. The effectiveness of drug immobilization was evaluated using an arbitrary scale of 1 to 3, from unsatisfactory immobilization and resistance to handling (category 1) to complete immobilization and superficial anesthesia (category 3).

A repeated measures analysis of variance (PROC GLM, SAS Institute, 1988) was used to test for relationships and interactions between independent variables (IV and IM routes of injection), and dependent variables (heart rate, respiratory rate, and core temperature) both within females (over time), and between females. Since no significant effect of time of immobilization on the dependent variables was de-

tected, only mean values for each female were used in further analyses. We used *t*-tests (PROC TTEST, SAS Institute, 1988) to evaluate differences in mean induction time, mean time to recovery, mean heart and respiratory rates, and mean core temperatures between routes of injection. All values are presented as mean  $\pm$  SD.

## RESULTS

Ketamine and diazepam induction dosages were  $3.64 \pm 1.3$  mg/kg and  $0.12 \pm 0.07$  mg/kg, respectively. Induction time and time to recovery for females injected IM ( $15 \pm 7$  min and  $47 \pm 16$  min, respectively) were significantly greater ( $t = 6.446$ ,  $P < 0.001$  and  $t = 3.425$ ,  $P < 0.01$ , respectively) than for females injected IV ( $0.6 \pm 0.4$  min and  $26 \pm 11$  min, respectively).

Of the 22 females immobilized, 15 were completely immobilized (category 3), and seven were partially immobilized (category 2). The IV route resulted in nine of 10 females completely immobilized, compared to six of 12 by the IM route. However, the mean ( $\pm$  SD) dosages of ketamine and diazepam for completely immobilized females were not significantly greater than for partially immobilized ones ( $3.59 (\pm 1.98)$  mg/kg and  $0.13 (\pm 0.005)$  mg/kg vs.  $3.41 (\pm 1.35)$  mg/kg and  $0.12 (\pm 0.005)$  mg/kg, respectively).

Generally, the first signs of drug effect were progressive ataxia and loss of coordination, decreased movement and sensitivity of flippers, muscle relaxation, and epiphora. Approximately 20 min after an IM or IV injection, minor to moderate tremors of the head, neck, and flippers were observed in some females. This side effect was most common on very hot days. No other adverse reactions, such as vomiting, were observed. Salivation was visibly decreased when atropine was used at a mean dosage of 0.004 mg/kg.

Heart rates in 22 immobilized seals were  $153 \pm 32$  beats/min, and ranged from 80 to 224 beats/min. Respiratory rates were  $6 \pm 3$  breaths/min, and ranged from two to 14 breaths/min ( $n = 22$ ). Core temperatures for the seals were  $37 \pm 0.91$  C, and

ranged from 35 to 38.9 C ( $n = 16$ ). Heart rates and core temperatures for females injected IV ( $173 \pm 15.71$  beats/min and  $37.6 \pm 0.83$  C) were significantly greater ( $t = 3.458$ ,  $P < 0.001$  and  $t = 2.208$ ,  $P < 0.04$ , respectively) than for females injected IM ( $135 \pm 27.1$  beats/min and  $36.5 \pm 1.15$  C). Respirations were regular, and rates were not affected by injection route ( $t = 0.07$ ,  $P > 0.05$ ). Independent of the injection route used, both heart and respiratory rates were significantly greater in 10 immobilized animals compared to 10 resting non-immobilized ones ( $91 \pm 14.3$  beats/min and  $3.1 \pm 0.73$  breaths/min;  $t = 5.85$ ,  $P < 0.001$  and  $t = 3.61$ ,  $P < 0.01$ , respectively).

Two fur seals died after being given 5.68 and 3.8 mg/kg of ketamine IM and 0.05 and 0.1 mg/kg of diazepam IM, respectively. In both cases, the animals were partially immobilized before death. One female exhibited a marked apnea 1 hr after the injection of the drugs and was administered 400 mg of doxapram hydrochloride IV, which rapidly reversed the apnea. However, respiration did not become regular and she died 2 hr later. The other fur seal initially was immobilized only with ketamine, but 15 min later exhibited generalized tremors. At that time she was administered 5 mg of diazepam IM, and died 30 min later. No significant lesions were observed during post mortem examination. Both females had given birth on the day of the capture.

Following immobilization, all females moved toward the sea, and many vocalized and smelled their pups soon after their release. Most of the females were observed with their pups for at least 2 days after their capture.

#### DISCUSSION

In adult female Juan Fernández fur seals, a combination of IM ketamine and diazepam produced mean induction and recovery times similar to values reported for ringed seals (*Phoca hispida*), harbor seals (*P. vitulina*), southern elephant seals (*Mir-*

*ounga leonina*), California sea lions (*Zalophus californianus*) and Galápagos sea lions (*Z. californianus wollebaeki*) administered ketamine IM (Geraci, 1973). The IM route is the most common injection route for ketamine and diazepam in pinnipeds (Gales, 1989), and offers the advantages of an easy and safe administration. The major disadvantage, particularly in remote delivery systems, includes drug injection into fat, resulting in delayed and reduced drug response, and variable and prolonged effect (Geraci, 1973). As expected, induction and duration of immobilization were significantly reduced when the IV route was used. Also, a much better degree of immobilization was attained. Engelhardt (1977) obtained similar results in harp seals, *Phoca groenlandica*, given ketamine IV. Despite these advantages, the IV use of ketamine and diazepam in Juan Fernández fur seals also resulted in a significant tachycardia and hyperthermia compared to the IM route.

The increased tachycardia and hyperthermia observed in immobilized seals differed from reports for other species of pinnipeds, in which ketamine was reported to cause minimal effects on the cardiovascular and thermoregulatory systems (Geraci et al., 1981; Parry et al., 1981). Our study differed from the others in the degree of activity and excitation of the animals prior to drug injection. In other studies, drugs were administered to resting or unalarmed animals either by using a remote method or by working with acclimatized captive seals. In our study, animals were chased before injection, resulting in sympathetic nervous system stimulation with a consequent increase in heart rate.

Despite the fact that pinnipeds are particularly susceptible to becoming overheated on land because of their high insulation (Hammond and Elsner, 1977), the problem of hyperthermia in phocids and otariids undergoing ketamine immobilization has been poorly addressed. Hyperthermia is a side effect of ketamine, and is likely to occur if quivering and muscle

tremors appear (Fowler, 1978). Based on our results, we believe that animals immobilized with ketamine are likely to suffer from hyperthermia, particularly after an intravenous injection. Furthermore, hyperthermia may have been the cause of death of one of the females in which core temperature increased 2 C in less than 20 min after generalized muscular tremors had appeared.

In humans, the effects of ketamine on the respiratory system are minimal and range from mild respiratory stimulation to mild depression (Fragen and Avram, 1989). In phocids, difficulties in breathing have been reported only with dosages over 6.86 mg/kg of ketamine (Engelhardt, 1977; Gales and Burton, 1987, 1988). In our study, signs of respiratory distress were observed only once, and respiration was regular throughout the immobilization in all other cases. The exception was a female with a marked apnea 1 hr after the injection of 5.68 mg/kg of ketamine IM and which died 2 hr later.

Ketamine was effective in achieving complete immobilization with dosages ranging from 2.16 to 6.76 mg/kg (mean of 3.59 mg/kg) when it was combined with diazepam at dosages ranging from 0.04 to 0.27 mg/kg (mean of 0.13 mg/kg). The degree of immobilization, however, was not related to the dose of drugs administered and higher dosages were not necessarily associated with better levels of immobilization. This individual variation in response to ketamine has occurred in other species of pinnipeds and has been attributed to factors such as accidental injection of drug into the blubber, inadequate circulation in peripheral vessels, age, disease, and level of excitation of the animals prior to injection (Geraci, 1973; Trillmich and Weisner, 1979).

We recommend the following to improve the use of ketamine and diazepam in Juan Fernández fur seals. A remote injection technique such as a blowpipe (Trillmich and Weisner, 1979; Parry et al., 1981) could decrease the level of excitation prior

to injection, thus minimizing side effects associated with the use of ketamine HCl. The intravenous route may be useful when rapid induction and recovery are required. In these cases, dosages should be lowered and heart rate as well as core temperature carefully monitored throughout the immobilization. The physiological state of the animals should be considered prior to immobilization, and ketamine HCl and diazepam should be used with caution in females with pups of less than 24 hr of age.

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