



Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*

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Received 21 October 2001; received in revised form 30 April 2002; accepted 11 June 2002

Abstract

The present experiments were designed to determine the efficacy of metomidate hydrochloride as an alternative anesthetic with potential cortisol blocking properties for channel catfish *Ictalurus punctatus*. Channel catfish (75 g) were exposed to concentrations of metomidate ranging from 0.5 to 16 ppm for a period of 60 min. At 16-ppm metomidate, mortality occurred in 65% of the catfish. No mortalities were observed at concentrations of 8 ppm or less. The minimum concentration of metomidate producing desirable anesthetic properties was 6 ppm. At this concentration, acceptable induction and recovery times were observed in catfish ranging from 3 to 810 g average body weight. Plasma cortisol levels during metomidate anesthesia (6 ppm) were compared to fish anesthetized with tricaine methanesulfonate (100 ppm), quinaldine (30 ppm) and clove oil (100 ppm). Cortisol levels of catfish treated with metomidate and clove oil remained at baseline levels during 30 min of anesthesia ($P > 0.05$). Plasma cortisol levels of tricaine methanesulfonate and quinaldine anesthetized catfish peaked approximately eight- and fourfold higher ($P < 0.05$), respectively, than fish treated with metomidate. These results suggest that the physiological disturbance of channel catfish during routine-handling procedures and stress-related research could be reduced through the use of metomidate as an anesthetic.

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Keywords: Anesthetic; Catfish; Cortisol; Metomidate; *Ictalurus punctatus*; Stress

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1. Introduction

Anesthetics are commonly used in aquacultural management and experimental procedures to ease handling and reduce fish stress. In addition to preventing physical injury, certain anesthetics reduce or block activation of the hypothalamo-pituitary-interrenal (HPI) axis associated with handling stressors. Failure to suppress stress-induced activation of the HPI axis results in a release of cortisol which in turn causes various physiological responses, the purpose of which is to help the fish overcome or compensate for the stress. Severe or chronic stress is often associated with poor performance and has long been associated with immunosuppression in cultured fish (Pickering and Duston, 1983; Thomas and Lewis, 1987; Maule et al., 1989).

Currently, tricaine methanesulphonate (TMS, MS-222) is the only US Food and Drug Administration (FDA) approved anesthetic for foodfish in the United States. Even when fish are deeply anesthetized with TMS, handling procedures activate the HPI axis, as evidenced by increases in circulating plasma cortisol concentrations (Pickering et al., 1982; Thomas and Robertson, 1991). Metomidate (DL-1-(1-phenylethyl)-5-(methoxycarbonyl) imidazole hydrochloride), marketed as a fish anesthetic under the trade name Marinil, is a rapid-acting nonbarbiturate hypnotic with potential cortisol suppressing properties. After 1 h of metomidate anesthesia, Olsen et al. (1995) observed insignificant cortisol increases in Atlantic salmon *Salmo salar* injected with adrenocorticotrophic hormone (ACTH) when compared to saline-injected salmon. On the contrary, ACTH-injected Atlantic salmon anesthetized with TMS exhibited plasma cortisol levels as high as 238 ng/ml. Thomas and Robertson (1991) demonstrated that after 15 min of continuous exposure to TMS or quinaldine, red drum *Sciaenops ocellatus* exhibited dose-related increases in plasma cortisol and glucose levels, whereas no changes in circulating cortisol or glucose were observed in metomidate-anesthetized fish.

The purpose of the present study was to determine the efficacy of metomidate as an anesthetic for channel catfish *Ictalurus punctatus*, and whether metomidate anesthesia suppressed the normal plasma cortisol increase associated with handling stress in this species. We report the stages of anesthesia associated with exposure to a range of metomidate concentrations, the minimum optimal anesthetic dose, and the effects of metomidate anesthesia on cortisol responsiveness to handling stress in comparison with that from anesthesia with TMS, quinaldine or clove oil.

2. Materials and methods

2.1. Anesthetics

Metomidate hydrochloride was acquired from Janssen Pharmaceutica, Beerse, Belgium. Tricaine methanesulphonate and quinaldine were purchased from Argent Chemical Laboratories (Redmond, WA, USA). Clove oil was purchased from Sigma (St. Louis, MO, USA).

2.2. Experiment 1

Channel catfish (USDA 103 strain; Wolters et al., 2000) were maintained in 72-l aquaria supplied with well water (temperature, 26 °C; pH, 8.6; total hardness, 120 ppm; alkalinity, 410 ppm; total ammonia nitrogen, 1.5 ppm; nitrite nitrogen, 0 ppm) at a flow rate of 8 l/min for the duration of this experiment. Two weeks prior to sampling, juvenile catfish (mean weight = 75 g) were stocked into 14 aquaria at 10 fish per aquaria. Fish were fed a 36% protein, floating, catfish fingerling starter (Farmland Industries, Kansas City, MO, USA) daily to satiety during the 2-week acclimation period, and photoperiod was held constant at 12-h light/12-h dark. All fish were fasted the day prior to sampling.

For the determination of efficacy, catfish were netted from the aquaria and transferred to a 100-l holding tank containing metomidate in 20-l well water with continuous aeration. Two aquaria of catfish were observed separately for each dose. The fish were held in the test solutions for 60 min regardless of anesthetic stage (Table 1). The criteria for determining efficacy were based on behavioral responses described by Schoettger and Julin (1967) and modified by Schoettger and Julin (1969). In the present study, an optimum anesthetic dose for general fish handling was established as the minimum dose producing the desired effects of rapid immobility without medullary collapse and rapid recovery. Rapid immobility was defined as a total loss of equilibrium (Stage 3) in all fish within 3 min. Concentrations of metomidate tested were 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 16.0 ppm. Following application of the anesthesia, the catfish were returned to the glass aquaria with flowing water and aeration, and observed to determine recovery time and survival. Feed was offered the following morning, and the fish were observed over the next week.

2.3. Experiment 2

Thirty USDA 103 strain channel catfish covering a range of 0.5–810 g average ($n=6$) body weight (fry, 0.5 ± 0.1 g; fingerling, 3.4 ± 0.4 g; juvenile, 20.9 ± 2.6 g; sub-adult, 508.0 ± 15.0 g; and adult, 810.0 ± 52.9 g) were acclimated in five tanks containing flowing, 26 °C well water for a minimum of 1 week. Feeding and environmental conditions were as described in Experiment 1. To determine the influence of channel catfish body weight on the efficacy of metomidate anesthesia, the six fish per size range were netted and transferred to a 100-l tank containing a 6-ppm metomidate solution in 20-l well water with continuous aeration. The fish were held in the anesthetic solution for 60 min. Time to reach Stage 3 (induction time), stage of anesthesia after 60 min, recovery time and survival were determined relative to body weight.

Table 1

Stages of anesthesia used to determine the efficacy of metomidate as an anesthetic for channel catfish (modified from Schoettger and Julin, 1969)

Stage 1	Sedation: decreased reactivity to external stimuli
Stage 2	Partial loss of equilibrium; erratic swimming
Stage 3	Total loss of equilibrium; cessation of locomotion
Stage 4	Medullary collapse

2.4. Experiment 3

The effect of metomidate anesthesia on circulating cortisol concentrations of anesthetized channel catfish was investigated and compared with TMS, quinaldine and clove oil anesthesia. Two weeks prior to sampling, USDA 103 strain channel catfish (mean weight = 75 g) were stocked at 10 fish per aquaria into sixteen 72-l aquaria, maintained and fed as in Experiment 1. All fish were fasted 1 day prior to sampling. To determine baseline plasma cortisol concentrations, 10 catfish were anesthetized with either 16 ppm metomidate, 200 ppm TMS, 70 ppm quinaldine, or 200 ppm clove oil, respectively, by adding anesthetic directly to an aquarium. Following the rapid induction of anesthesia at these doses, six fish per treatment were immediately collected and bled from the caudal vasculature using heparinized syringes. Plasma was collected by centrifugation at 4 °C and stored at –20 °C for subsequent cortisol analysis. Circulating cortisol concentrations during anesthesia at optimal doses were determined by quickly netting catfish from three aquaria and transferring them to a 100-l tank containing 20-l of well water and either 6 ppm metomidate, 100 ppm TMS (Schoettger and Julin, 1967), 30 ppm quinaldine (Schoettger and Julin, 1969), or 100 ppm clove oil (Waterstrat, 1999), respectively. No additional buffering was done for any of the treatments. The 100-ppm TMS anesthetic bath had a pH of 8.1. Blood was collected from six fish every 10 min for 30 min. Blood collection and storage were conducted as described above. Plasma cortisol concentrations were determined by time-resolved fluoroimmunoassay (TR-FIA) validated for channel catfish plasma (Small and Davis, 2002).

Plasma cortisol concentrations were subjected to factorial analysis of variance (ANOVA) mixed-model procedures using the SAS software system version 8.00 (SAS Institute, Cary, NC, USA). Pairwise contrasts were used to identify significant differences at the 5% level among anesthetics at different time points. Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values, and the Shapiro–Wilkes test for normality.

3. Results

3.1. Experiment 1

Channel catfish were exposed to concentrations of metomidate anesthesia ranging from 0.5 to 16 ppm (Table 2). At concentrations of 2 ppm or less, Stage 3 anesthesia was not observed in any catfish after 3-min exposure time, nor did they reach Stage 3 within the 60-min exposure period. When treated with 4 ppm metomidate, 60% of the channel catfish reached Stage 3 within 3 min, and 100% of the fish exposed to this concentration reached Stage 3 within 8 min of exposure. Concentrations of 6-, 8- and 16-ppm metomidate resulted in 100% induction of Stage 3 anesthesia within 3 min; however, 90% of the fish treated with 16-ppm metomidate reached Stage 4 prior to 5 min of exposure. Following 60 min of metomidate exposure, recovery time in fresh water was rapid (<3 min) among catfish treated with 8 ppm or less, and survival was 100%. Only 35% of the fish exposed to 16-ppm metomidate survived. Those fish surviving 16 ppm had a recovery time of over

Table 2

Efficacy of several concentrations of metomidate on anesthetizing 75-g channel catfish to total loss of equilibrium (Stage 3) within 3 min, and recovery, stage and survival after 60-min exposure

Concentration (ppm)	Fish in Stage 3 anesthesia (%)	Recovery time ^a (min)	Stage of anesthesia after 60 min	Survival (%)
0.50	0	n/a	1	100
1.0	0	n/a	1	100
2.0	0	n/a	1	100
4.0	60	0.65 ± 0.06	3	100
6.0	100	1.35 ± 0.12	3	100
8.0	100	2.01 ± 0.11	3	100
16.0	100	20.32 ± 2.27	4	35

^a Mean ± SE ($n=20$).

20 min and did not feed during the week after anesthetization. All fish treated with 8 ppm or less began feeding within 2 days following treatment.

3.2. Experiment 2

In order to determine the effect of body weight on metomidate anesthetic efficacy, six catfish of each size range were anesthetized with 6-ppm metomidate for a period of 60 min (Table 3). All the catfish, regardless of body weight, reached Stage 3 anesthesia within 3 min. Catfish averaging 0.5 g body weight had either sporadic or no observable gill movement; however, 100% of the catfish recovered rapidly and survived the treatment.

3.3. Experiment 3

Comparison of circulating plasma cortisol levels in channel catfish anesthetized with metomidate, TMS, quinaldine or clove oil resulted in significant differences among treatments over time (Fig. 1). Baseline plasma cortisol levels were similar regardless of the anesthetic used. After 10 min of exposure, catfish treated with TMS had significantly higher plasma cortisol concentrations than those treated with quinaldine, clove oil or metomidate. Plasma cortisol concentrations of TMS-treated fish remained significantly higher than those in other treatments ($P<0.05$) over the entire 30 min exposure. Catfish treated with quinaldine also demonstrated elevated ($P<0.05$) cortisol levels when

Table 3

Time to Stage 3 anesthesia (induction time), stage of anesthesia, recovery time and survival of channel catfish across a variety of body weights during a 60-min immersion in a 6-ppm metomidate bath

Mean weight ^a (g)	Induction time ^a (min)	Stage of anesthesia after 60 min	Recovery time ^a (min)	Survival (%)
0.5 ± 0.1	0.40 ± 0.11	3–4	4.05 ± 0.54	100
3.4 ± 0.4	0.54 ± 0.08	3	2.76 ± 0.24	100
20.9 ± 2.6	1.00 ± 0.08	3	2.08 ± 0.23	100
508.0 ± 15.0	2.66 ± 0.45	3	2.43 ± 0.80	100
810.0 ± 52.9	2.08 ± 0.23	3	1.74 ± 0.27	100

^a Mean ± SE ($n=6$).

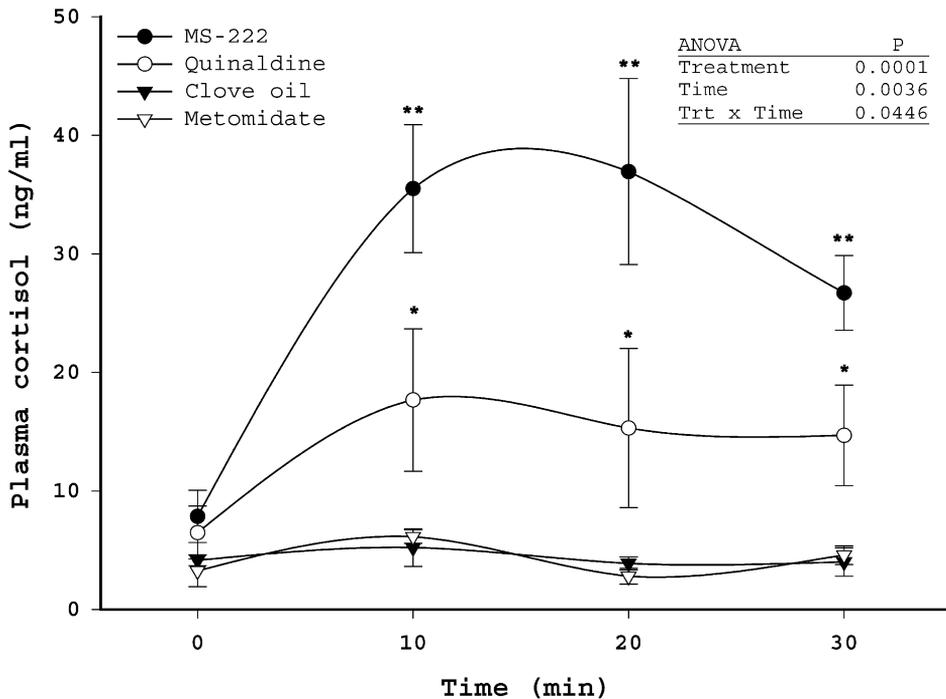


Fig. 1. Mean (\pm SE) plasma cortisol concentrations of channel catfish during 30-min anesthetization with 100 ppm TMS, 30 ppm quinaldine, 100 ppm clove oil or 6 ppm metomidate ($n=6$). Asterisks indicate significant differences between treatments at a given time point.

compared to clove oil- and metomidate-treated fish. Plasma cortisol levels of catfish anesthetized with metomidate or clove oil did not change throughout the 30-min exposure.

4. Discussion

Metomidate is the methyl derivative of etomidate. Both are rapid-acting, nonbarbiturate hypnotics with demonstrated capacity for reducing plasma corticosteroid concentrations (Preziosi and Vacca, 1982; Fraser et al., 1984; Thomas and Robertson, 1991; Olsen et al., 1995). Limsuwan et al. (1983) found that catfish anesthetized with 3 ppm etomidate and then confined for 10 min had reduced cortisol levels compared to unanesthetized fish. Although the mechanisms of metomidate action on cortisol synthesis have not been well established, etomidate has been demonstrated to affect the mitochondrial cytochrome P₄₅₀-dependant enzymes that catalyze the synthesis of cortisol (Vanden Bossche et al., 1984; Wagner et al., 1984).

The first part of this study was to evaluate the efficacy of metomidate as an anesthetic for catfish, and establish the minimum dose producing desirable anesthetic properties. Desirable anesthetic properties for finfish were defined by Schoettger and Julin (1967) while investigating the efficacy of TMS as an anesthetic for salmonids. These authors

described two levels of anesthetic use and associated desirable effects. For the desired effects of rapid immobility, rapid recovery and brief immersion time, as might be required during handling, they site the criteria for effective concentration as that which produces loss of reflex in all fish within 3 min. Schoettger and Julin (1969) modified these criteria as a result of differences in behavioral responses of fish anesthetized with quinaldine, incorporating the loss of equilibrium and cessation of locomotion in place of loss of reflex. Behavior of catfish anesthetized with metomidate also differs from that of catfish exposed to TMS. Metomidate anesthesia progresses rapidly to a total loss of equilibrium (Stage 3) without entering a prolonged stage of sedation. Catfish anesthetized in 6-ppm concentrations of metomidate elicited small reflex movements when the container was struck, which were much less severe than those associated with quinaldine, but the fish were easily handled. In experiments with rainbow trout *Oncorhynchus mykiss* (Gilderhus and Marking, 1987), cod *Gadus morhua* (Mattson and Ripple, 1989), and Atlantic salmon (Olsen et al., 1995), fish were “handleable” within 3 min of metomidate anesthesia. Catfish between 3 and 810 g average body weight all reached Stage 3 and all recovered in less than 3 min. While all 0.5-g catfish survived 6-ppm metomidate exposure for 60 min, a lower dose may be preferable due to the extremely sporadic, even unobservable, opercular movement in these small fish.

The second part of this study investigated the effect of metomidate anesthesia on circulating plasma cortisol levels of channel catfish, and compared these results to catfish anesthetized with TMS, quinaldine and clove oil. The results showed that the short duration of handling associated with moving catfish into anesthesia solution and bleeding anesthetized fish evoked a cortisol stress response. This response is dependent upon and possibly exacerbated by the type of anesthetic used. In this study, catfish anesthetized for 10–20 min with TMS had approximately an eightfold increase in plasma cortisol concentrations, and catfish anesthetized with quinaldine had approximately a fourfold increase above baseline cortisol concentrations. In contrast, metomidate was effective at suppressing the cortisol stress response and maintaining baseline concentrations. This finding was not unexpected since metomidate has been shown to suppress the corticosteroid response in other fish by blocking the actions of ACTH (Thomas and Robertson, 1991; Olsen et al., 1995). Of interest, is the observation that clove oil also suppressed plasma cortisol levels in the present study. This result was unexpected, and warrants further investigation with channel catfish. Clove oil appears to be a viable anesthetic for aquacultural use (Taylor and Roberts, 1999), and is efficacious as an anesthetic for channel catfish (Waterstrat, 1999).

Aquaculture inherently involves stressing fish. Handling, transportation, poor water quality and crowding are common stressors in fish culture, resulting in poor overall performance, disease and even mortality. Thus, for farmers, fish stress can result in a substantial economic loss, and for researchers, stress can confound experimental results. Anesthetics provide a useful means of reducing physical damage and preventing the exacerbation of handling stress. Metomidate effectively suppresses the cortisol stress response, and may prove to be a useful anesthetic for reducing the adverse effects of stress. As counter-point, Thomas and Robertson (1991) suggest that an “adequate” corticosteroid stress response may be essential for recovery from severe or prolonged stressors, citing Ledingham and Watt (1983) as reporting increased post-surgery mortality in patients

anesthetized with metomidate. While under extreme research conditions this point may be valid, typical husbandry and handling procedures would not likely result in such a high degree of stress.

5. Conclusion

In summary, metomidate is a potent anesthetic for channel catfish, having both rapid induction and recovery times. The minimum desirable concentration for anesthesia, that which resulted in a total loss of equilibrium in all fish within 3 min, was determined to be 6 ppm. This concentration proved to be effective for catfish ranging from 3 to 810 g average body weight. Metomidate anesthesia was found to suppress the cortisol stress response in channel catfish, and is well suited for stress studies and procedures requiring suppression of circulating cortisol levels.

Acknowledgements

The authors wish to acknowledge the technical assistance of Ms. Priscilla Barger and Mr. Jimmie Warren of the USDA/ARS Catfish Genetics Research Unit, and Dr. Hank Stoddard of Shamrock Veterinary Clinic (Cross City, FL, USA) for acquiring the metomidate for purchase. Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply approval to the exclusion of other products that may be suitable.

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