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THE EFFECTS OF PROPISCIN (ETOMIDATE) ON THE BEHAVIOUR, HEART RATE, AND VENTILATION OF COMMON CARP, *CYPRINUS CARPIO* L.

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Background. The use of anaesthetics in aquaculture requires conducting specific studies of their effects on fish. The objective of this study was to evaluate the effects of Propiscin (Polish agent containing etomidate) on common carp behaviour, heart rate, and ventilation.

Material and methods. Carp were anaesthetised with Propiscin at the concentrations of 1 and 2 $ml \cdot l^{-1}$. An electrocardiograph was used to record the heart rate and the amplitude and rate of the opercular movements. The effects of atropine and forced water flow over the gill system on the anaesthetised carp's heart rate were also studied.

Results. At either concentration, Propiscin caused general anaesthesia manifesting itself in: a loss of equilibrium, faded responses to external stimuli, gradual ventilation failure, and bradycardia. A high correlation between respiration rate and heart rate was found in the anaesthetised fish.

Conclusion. The hypoxia was the direct cause of bradycardia, and the regularity and amplitude of breathing were the most important indicators of the level of Propiscin-evoked anaesthesia.

Key words: fish, anaesthesia, Propiscin, etomidate, heart rate, ventilation, common carp.

INTRODUCTION

Anaesthetics are used in aquaculture to facilitate various routine handling procedures, such as weighing, sorting, collection of spawning material, tagging or veterinary treatments (Tytler and Hawkins 1981, Brown 1987). The most widely used anaesthetics include MS-222 (tricaine methanesulphonate), benzocaine, etomidate, metomidate, phenoxyethanol, quinaldine, and quinaldine sulphate (Gomułka and Antychowicz 1999). In Poland, Propiscin has recently become a commonly used agent.

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Anaesthetics act with various intensity, driving fish into general anaesthesia resulting in loss of consciousness, loss of pain sensation, inhibition of reflex activity, and reduced skeletal muscle tone. The depth of anaesthesia can be assessed trough changes in the mobility, response to external stimuli, ability to regain equilibrium, ventilation rate, and the muscle tone (Gomułka and Antychowicz 1999). Regardless of the agent, the process of anaesthesia in fish, develops in a similar way and runs in a progressive pattern (McFarland 1959). Overdosing with an anaesthetic or retaining the fish in anaesthetic solution for too long leads to suffocation and death (Siwicki 1984).

Although numerous studies of fish anaesthetics have been carried out, most of them dealt with the determination of effective dosages and lethal concentrations.

Studies that have assessed the physiological effects of anaesthesia have been concerned mostly with haematological analyses. Relatively little has been done on the physiological effects of particular agents on basic functions like circulation and respiration.

This paper presents the results of our studies on the effects of Propiscin, a new yet commonly used general anaesthetic for fish (Kazuń and Siwicki 2001), on common carp. The process of anaesthesia with this agent was analysed based on both behavioural responses of the fish and on simultaneous electrocardiographic records of their heart function and ventilation rate.

MATERIAL AND METHODS

The experiments were carried out using 38 common carp with average weight of 254.8 ± 73.4 g. The fish were obtained from a cage culture facility at Gryfino near Szczecin, Poland. The fish were acclimated for 14 days in a 1000-l common tank prior to experimentation, in aerated tap water at 20 ± 1°C.

The following chemicals were applied in the experiments:

- Propiscin, a short-term general anaesthetic for fish, which contains etomidate (0.2%) as the active substance, prepared by the Division of Fish Immunology and Pathology in Żabieniec, Inland Fisheries Institute in Olsztyn, Poland.
- Atropine (1% atropinum sulfuricum), manufactured by the Warsaw Pharmaceutical Factory "Polfa".

Cardiac and respiratory rates were recorded with an Ascard B5 electrocardiograph. The ECG record was obtained from a bipolar output, with electrodes placed in a fish's body based on the procedure described by Labat (1966). Isolated copper wires (1-mm diameter) were used as the electrodes, with the isolation removed along 5 mm, 7 cm from their free ends.

In order to implant the electrodes, the fish were anaesthetised in a Propiscin solution of 2 $ml \cdot l^{-1}$, until showing no response to stimuli. Thereafter, the fish were transferred to a solution of 0.5 $ml \cdot l^{-1}$, and the electrodes implanted. During the recovery, fish were placed in a darkened, 18-l experimental tank with aerated and filtered water.

The rate of the ventilatory movements was recorded using an additional electrode fixed to the edge of the operculum. The neutral electrode was immersed into the water. The positioning of the electrodes is shown in Fig. 1, and an example of ECG record is presented in Fig. 2.

Experimental protocol

1. An analysis of the effect of Propiscin at a concentration of 1 $ml \cdot l^{-1}$ on heart and ventilatory rates. Time of exposure: 14 min. After electrode implantation, each fish was placed in an experimental tank. When the fish regained normal behaviour, control records of cardiac action and respiration rate were taken for another 60 min. Thereafter, a solution of Propiscin was administered to the water to reach the desired concentration. The first record took place in 0.5 min after the Propiscin administration, while subsequent records were repeated every 1 minute. After 14 minutes, fish were transported to a tank with clean, aerated water and recording continued until the fish regained full movement activity. The experiment was carried out on 7 individuals.

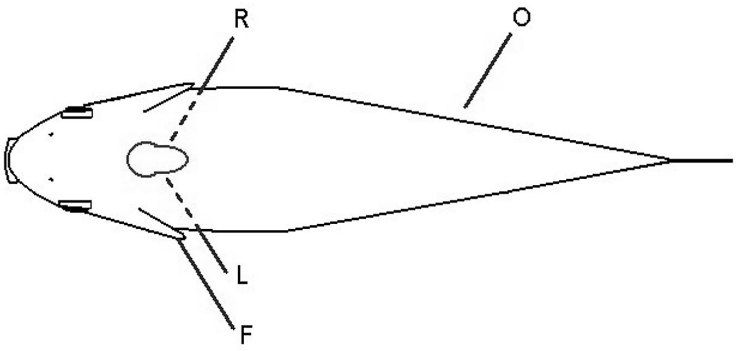

Fig. 1. Positioning of measuring electrodes; R and L, electrodes for recording heart function; F, electrode for recording opercular movements; O, neutral electrode

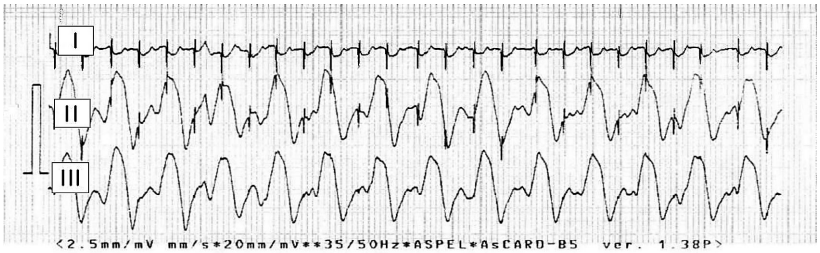

Fig. 2. Record of bioelectric heart action and ventilatory movements of carp; I, heart action record; II, heart action record and opercular movements; III, opercular movements

2. An analysis of the effect of Propiscin at a concentration of 2 $ml \cdot l^{-1}$ on heart and ventilatory rates. Time of exposure: 7 min; 1-h adaptation period. The experiment was carried out on 7 carp.
3. An analysis of the effect of Propiscin at a concentration of 2 $ml \cdot l^{-1}$ on heart and ventilatory rates. Time of exposure: 7 min; 12-h adaptation period. The experiment was started 12 hours after the implantation of electrodes. It was carried out on 13 carp.
4. An analysis of the effect of atropine on the anaesthetic effect of Propiscin (2 $ml \cdot l^{-1}$). Time of exposure: 7 min. The anaesthesia was preceded by a pericardial injection of 0.4-ml solution of atropine (0.4 mg) 1 hour before the administration of Propiscin. The experiment was carried out on 7 carp.
5. An analysis of the effect of forced water flow over the gill system on the heart rate of the carp anaesthetised with Propiscin (2 $ml \cdot l^{-1}$). The fish were kept in a Propiscin solution (2 $ml \cdot l^{-1}$) until respiratory movements ceased. Next, the anaesthetic solution was made to flow over the gills using a tube placed in the fish's mouth. The experiment was carried out in 3 replications on 4 fish.

The recorded data were subjected to statistical analysis using Statistica software. The significance of differences between the values obtained within each minute of the experiments and the control values was estimated with Wilcoxon's matched pairs rank test. The control values between particular experiments were compared using the Mann–Whitney test. Pearson's coefficients of correlation (r) were calculated in order to estimate the degree of correlation between the heart and ventilatory rhythms. A confidence level of 95% ($\alpha = 0.05$) was used.

The presently described studies were carried out within 1998–1999, when no authorisation was needed for experiments on animals. Similar studies, however, carried out later by the same research team were approved by the Local Ethical Committee for Experiments on Animals.

RESULTS

The administration of Propiscin at concentrations of 1 and 2 $ml \cdot l^{-1}$ resulted in progressive anaesthesia. Its first symptom was sedation, followed by loss of equilibrium and gradually deepening general anaesthesia accompanied by slowing and fading of the ventilation.

After transfer to a tank with clean water, all the fish recovered from the anaesthesia. Its symptoms disappeared in reverse order, beginning with the return of ventilation.

Experiment 1

Before the anaesthetic had been administered to the experimental tank, the fish were swimming restlessly. Propiscin caused sedation and equilibrium disturbance. Total loss of equilibrium was observed in 3.4 ± 1.2 min on average. The fish regained their vertical position 16.7 ± 7.9 min on average after the exposure.

The changes in the rates of heartbeat and breathing observed during the anaesthesia are presented in Fig. 3. As a result of Propiscin administration to water, the fish examined showed parallel reductions in the rates of respiration and circulation.

Placing the fish in the tank with clean water resulted in a reversed process; the rates of heart action and ventilation gradually increased to the levels close to the control. The correlation coefficient between respiration and heart rates was 0.95, which revealed a strict relationship between these functions.

Experiment 2

Before the anaesthetic had been administered, the fish swam restlessly. Propiscin caused sedation and equilibrium disturbance. Total loss of equilibrium was observed in 2.4 ± 0.7 min on average. Regaining the vertical position was observed in 20.2 ± 13.6 min after the exposure.

The changes in the rates of heart action and breathing observed during the anaesthesia are presented in Fig. 4.

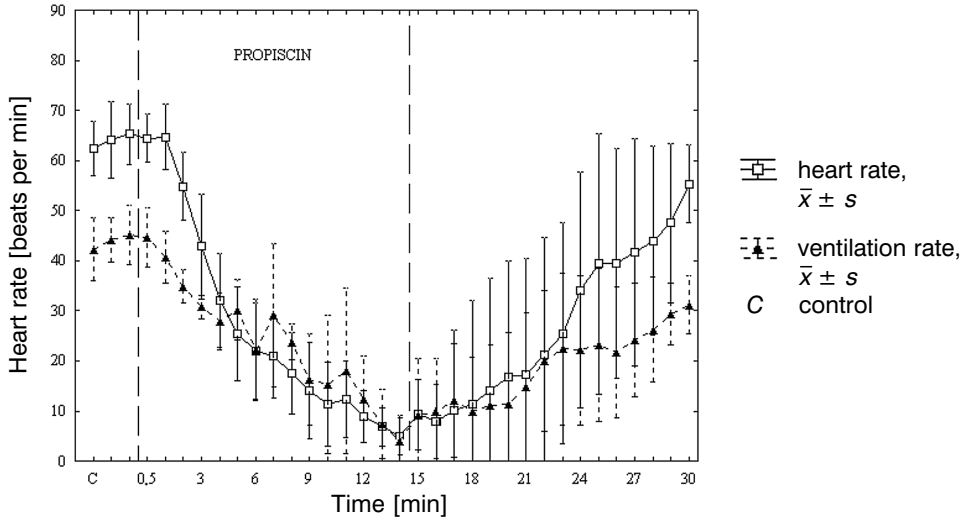

Fig. 3. The effect of Propiscin at a concentration of 1 $ml \cdot l^{-1}$ of water on ventilatory and heart rates of carp

Experiment 3

Before the anaesthetic had been applied, most of the fish were quiet. Propiscin caused sedation and equilibrium disturbance. Sometimes, after the administration of the agent, a short-term rise in mobility was observed. Total loss of equilibrium was recorded in 2.5 ± 0.5 min, the fish returned to their vertical position between 14 and 80 (mean 31.4 ± 20.1) min after the exposure.

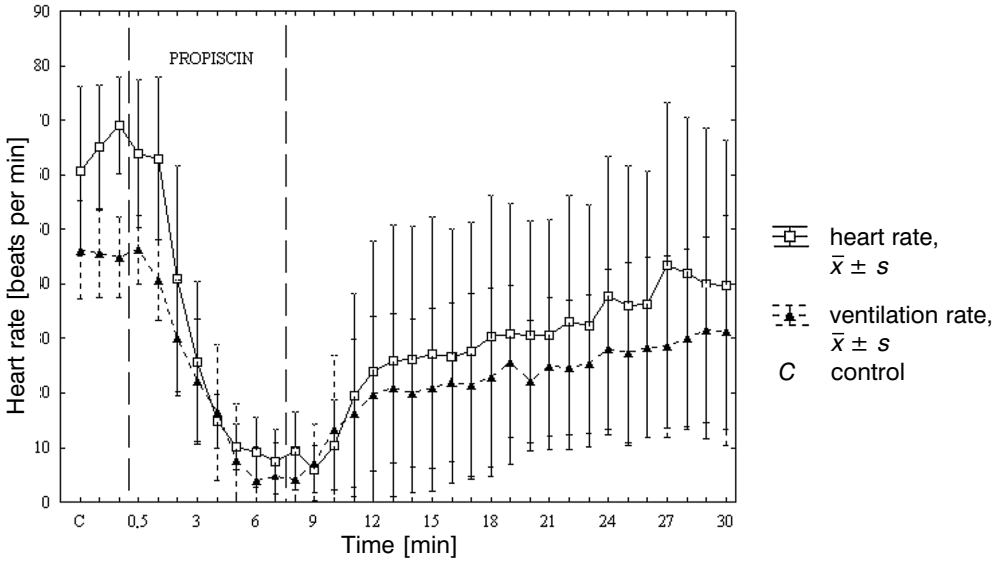

Fig. 4. The effect of Propiscin at a concentration of 2 ml·l^{-1} of water on ventilatory and heart rates of carp (the correlation coefficient between cardiac and respiration rates was 0.98)

The changes in the rates of heartbeat and respiration observed during the anaesthesia are presented in Fig. 5. The coefficient of correlation between ventilatory and cardiac rates was 0.95.

Experiment 4

Before the anaesthetic had been administered, the fish were swimming restlessly. Propiscin caused sedation and equilibrium disturbance. Total loss of equilibrium was observed in 2.1 ± 0.3 min of exposure. Return to a vertical position was recorded 20.2 ± 11.8 min after the exposure.

The application of Propiscin resulted in a slight, but significant ($P < 0.05$) reduction in heart rate and depression of ventilatory rate (Fig. 6).

The correlation coefficient (r) between respiration and cardiac rates was 0.66 and was the lowest among all the experiments.

The control values between particular experiments are compared in Table 1.

Experiment 5

The effect of forced water flow on the cardiac action in a carp anaesthetised with Propiscin.

The fading of opercular movements during anaesthesia was accompanied by acute bradycardia. The flow of water (containing the anaesthetic) forced through the gill system resulted in abrupt cardiac rate acceleration. Stopping the flow evoked the bradycardia. Fig. 7 presents a typical record of the carp's heart response to water flow.

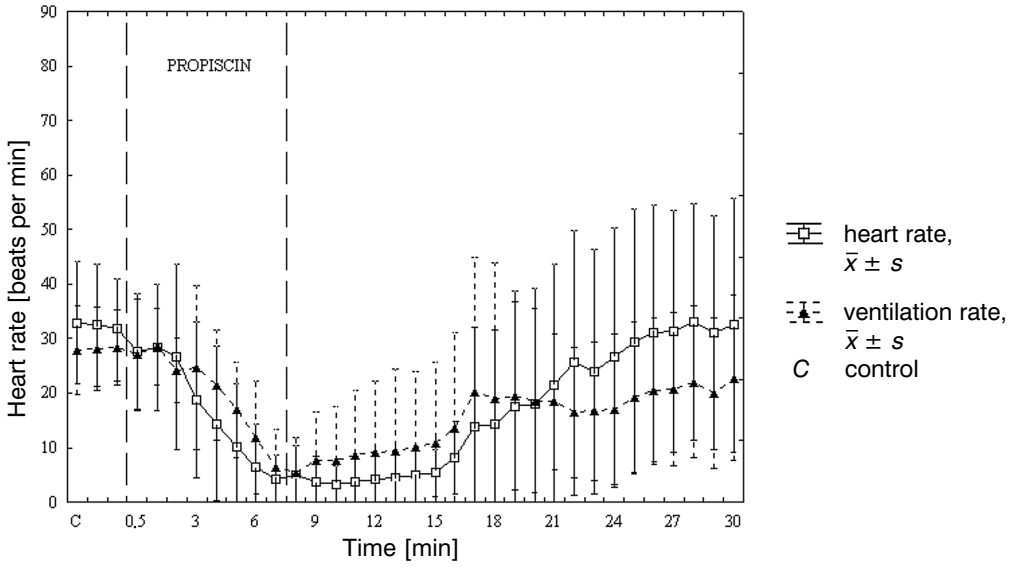

Fig. 5. The effect of Propiscin at a concentration of 2 ml·l^{-1}of water on ventilatory and heart rates of carp; the experiment followed a 12-hour period with implanted electrodes

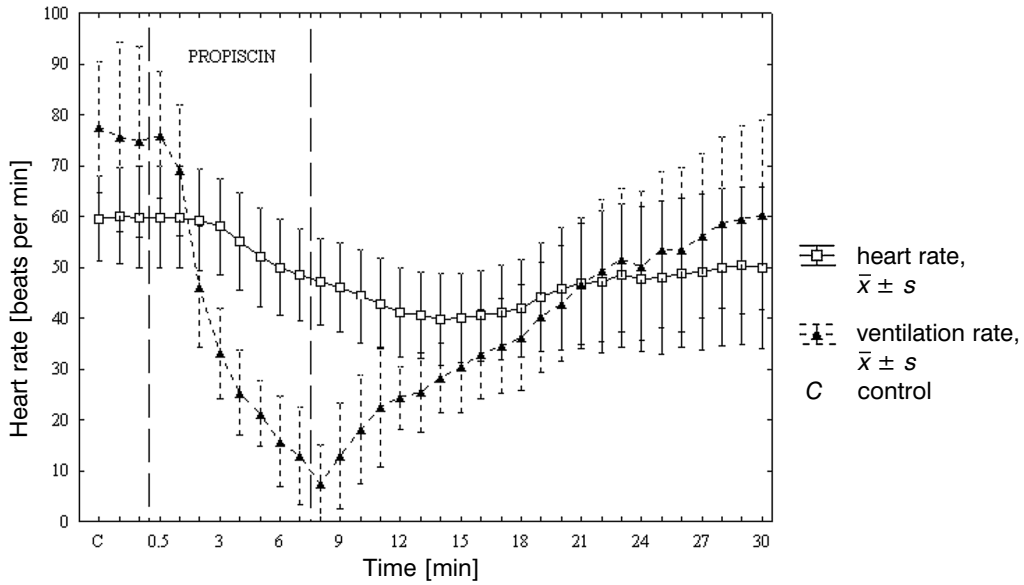

Fig. 6. The effect of Propiscin at a concentration of 2 ml·l^{-1}on ventilatory and heart rates of carp; the experiment was preceded by intrapericardial injection of atropine (0.4 mg)

Table 1

The comparison of control values between particular experiments (the Mann–Whitney test). The values significantly different are distinguished by bold type

Group 1	Group 2	rank sum 1	rank sum 2	U	Z	P	N_1	N_2
P1 H	P2 H	432.5	470.5	201.5	–0.47	0.63	21	21
P1 H	AT H	495.0	408.0	177	1.09	0.27	21	21
P1 H	**AD H**	**1048.0**	**782.0**	**2**	**6.31**	**<0.05**	**21**	**39**
P2 H	AT H	511.5	391.5	160.5	1.50	0.13	21	21
P2 H	**AD H**	**1035.5**	**794.5**	**14.5**	**6.12**	**<0.05**	**21**	**39**
AT H	**AD H**	**1021.5**	**808.5**	**28.5**	**5.90**	**<0.05**	**21**	**39**
P1 V	P2 V	434.0	469.0	203	–0.44	0.65	21	21
P1 V	**AT V**	**260.0**	**643.0**	**29**	**–4.81**	**<0.05**	**21**	**21**
P1 V	**AD V**	**1005.0**	**825.0**	**45**	**5.64**	**<0.05**	**21**	**39**
P2 V	**AT V**	**267.0**	**636.0**	**36**	**–4.64**	**<0.05**	**21**	**21**
P2 V	**AD V**	**1013.0**	**817.0**	**37**	**5.77**	**<0.05**	**21**	**39**
AT V	**AD V**	**1043.0**	**787.0**	**7**	**6.23**	**<0.05**	**21**	**39**

H, heart rate; V, ventilatory rate; P1, experiment with Propiscin at a concentration of 1 $ml \cdot l^{-1}$; P2, experiment with Propiscin at a concentration of 2 $ml \cdot l^{-1}$; AD, experiment with 12-hour adaptation period; AT, experiment with atropine.

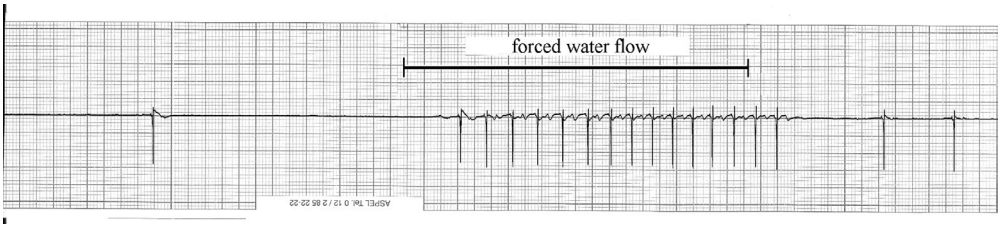

Fig. 7. Response of an anaesthetised carp's heart to water containing Propiscin (2 $ml \cdot l^{-1}$) forced to flow through the gill system

DISCUSSION

The experiments demonstrated that Propiscin caused reversible, gradual, parallel reduction in the heart and ventilation rates. The applied time of exposure often resulted in reduction of both rhythms down to drastically low levels (cardiac rate ⩽ 1 per minute and total apnoea). Similar effects of etomidate on heart rate in the spinally transacted rainbow trout, *Oncorhynchus mykiss*, were observed by Fredricks et al. (1993). Also closely related drug metomidate caused respiratory and heart rate depression in turbot, "*Scophthalmus maximus*" (valid name: *Psetta maxima*), and in Atlantic halibut, *Hippoglossus hippoglossus* (cf. Hansen et al. 2003).

Studies on the effects of other anaesthetics on cardiac function and respiration in fish show that each agent has a different impact. Serfaty et al. (1959) found that the administration of MS-222 to common carp resulted in elevated heart rate. Overdosing, manifested by respiratory failure, led to an atrioventricular block, acute arrhythmia, and bradycardia. A similar effect was described by Randall (1962) in tench, *Tinca tinca*, by Houston et al. (1971), in brook trout, *Salvelinus fontinalis*, and by Fredricks et al. (1993) in rainbow trout. Yamamitsu and Itazawa (1988) demonstrated that in fish under light 2-phenoxyethanol anaesthesia, the heart rate was higher than in controls. However, with increasing concentration, the duration of tachycardia shortened, giving way to progressive bradycardia accompanied by fading of the ventilatory movements. The depressive effect of this agent on heart rate was also observed by Fredricks et al. (1993) in rainbow trout.

Mitsuda et al. (1982), who investigated CO_2 anaesthesia effects on carp, recorded bradycardia accompanied by a slowed ventilation rate.

This demonstrates that the effects of anaesthetics on heart action and ventilation have a common element, which causes disturbances leading to the complete failure of both functions. Differences in those processes may result from different action mechanisms of particular agents as well as from the concentrations and other factors.

The effect of experimental environment

Under stress conditions, catecholamines are secreted to fish's blood (Nilsson et al. 1976, Wahlqvist and Nilsson 1980, Ling and Wells 1985), which may lead to increased cardiac and ventilatory rates (Wahlqvist and Nilsson 1977, Graham and Farrell 1989, Burleson and Milsom 1995). An acceleration of cardiac action caused by stress may also result from an enhanced activity of adrenergic nerve fibres (Holmgren 1977, Smith and Jones 1978, Axelsson and Nilsson 1986, Temma et al. 1986, Axelsson 1988). Therefore, the effect of the experimental environment on the fish may be concluded from changes in heart and ventilation rhythms.

The control values of heart rate (before administration of Propiscin) recorded in our studies (experiments 1 and 2) were relatively high, more than 60 beats per minute. The behaviour of the fish observed at that time revealed their excitation. Hence, it can be concluded that the applied time of 1 h between electrodes implantation and control values recording may have been too short for the fish to adapt to a relatively small experimental chamber and to the discomfort caused by the electrodes.

Significantly lower values of heart and ventilation rates before the anaesthesia were recorded in experiment 3, in which the period of adaptation was extended to 12 hours. This probably means that the 12-h span between placing the fish with the electrodes on in the experimental tank and the proper experiment allowed the animals to adapt to these new conditions, significantly reducing stress.

Also, the process of anaesthesia in carp treated with Propiscin (at 2 $ml \cdot l^{-1}$) 1 h after a short-term anaesthesia (needed for electrodes implanting) differed from the anaesthesia process in the fish that were anaesthetised with Propiscin 12 h after awakening from the implantation-related anaesthesia. During the anaesthesia following a 1-h adaptation (experiment 2), both heart and ventilation rates declined more rapidly and the anaesthetic state lasted shorter as compared with the fish re-anaesthetised after 12 hours (experiment 3).

The more rapid progress of anaesthesia if using Propiscin soon after the initial anaesthesia may have resulted from residuals of etomidate still present in blood. This conforms to the results reported by Serfaty et al. (1959), who observed fish's increased susceptibility to anaesthetic resulting from the additive effect of two consecutive anaesthetic treatments.

It seems that the earlier recovering of the fish which were re-subjected to Propiscin (within 1 h after the initial anaesthesia) observed in our studies may have resulted from a shorter period of a respiratory activity during the second exposure to Propiscin, as a result of which a lower quantity of etomidate penetrated the blood.

Anaesthesia preceded by atropine injection

Atropine was used to determine the role of cholinergic innervation of the heart in bradycardia appearing during the Propiscin-evoked anaesthesia. It blocks non-selectively the muscarine receptors and is efficient in preventing bradycardia caused by the activity of the vagus nerve (Gannon and Burnstock 1969, Saito 1973, Cobb and Santer 1973, Holmgren 1977). An intrapericardial injection of atropine solution is a verified method of evaluation of the cholinergic effect on fish's heart function (Randall 1966, Randall and Smith 1967, Smith and Davie 1984).

The control values of the heart rate observed in these experiments in the fish premedicated with atropine did not differ significantly from the control values of previous experiments, under which also a 1-h adaptation was applied. This indicates that under such conditions the cholinergic inhibition did not significantly influence the heart function. This can be explained by the increase of heart rate under stress and locomotory excitation due to an increased quantity of catecholamines in the blood and/or the enhanced activity of the sympathetic innervation of the heart with parallel reduction in the pressure of the parasympathetic side of the nervous system (Axelsson 1988, Axelsson and Nilsson 1986, Butler et al. 1986, Smith 1978, Wahlqvist and Nilsson 1977). However, Iwama et al. (1989) reported that the level of blood adrenaline in rainbow trout, during anaesthesia with five agents was the highest in the stage of opercular movements cessation and the lowest in the control and after recovery.

Our experiments demonstrated that in the process of anaesthesia atropine considerably reduced the depressive effect of Propiscin on the heart, preserving respiratory response. The observed reduction in heart rate was, however, still significant in relation to the control values.

It can be concluded, that cholinergic transmission may not have been entirely blocked. Besides the cholinergic one, another mechanism that could reduce the cardiac rate is also possible. As the reduced cardiac rhythm was anyway higher than the control levels arrived at in the experiment with the 12-h adaptation, it can be presumed that the decline in heart rate could have also resulted from a lower activity of the sympathetic system. The decreased activity of the sympathetic fibres as a result of etomidate injection was demonstrated by Hughes and MacKenzie (1978) in rabbits.

In the available literature, only mentions can be found that injecting atropine partially blocks bradycardia in live fish. In crucian carp, an atropine injection by 80 percentage points reduced the bradycardia caused by arrested water flow through the gill system, which was recognised as sufficient evidence that cholinergic fibres were underlying this inhibition (Randall 1966). A similar effect was observed in studies on trout (Randall and Smith 1967) and on piked dogfish, *Squalus acanthias* (cf. Satchell 1961), however, these authors did not analyse atropine efficiency.

The results of our experiments with atropine indicate that the main cause of Propiscin-caused bradycardia was an increased inhibitive effect of cholinergic fibres with potential involvement of other elements of the mechanism controlling the fish's heart function.

Experiments with forced water flow

In our studies, an interesting result was recorded when a forced anaesthetic-containing water flow through the mouth and gill system was applied for the anaesthetised fish. The experiments were started as soon as the respiratory movements had faded. As a nearly immediate response to water flow, the bradycardia ceased. Arresting the flow again resulted in a heart rate slowdown. A similar effect of forced water flow on the heart action during anaesthesia was reported by Houston et al. (1971), who anaesthetised *Salvelinus fontinalis* using MS-222, and by Wardle and Kanwisher (1974), who studied an effect of amylobarbitone on Atlantic cod, *Gadus morhua*.

A range of studies has demonstrated that insufficient water oxygenation, or a hindered flow through the gill system, results in bradycardia (Satchell 1961, Hoelton and Randall 1967, Marvin and Heath 1968, Marvin and Burton 1973). Oxygen and carbon dioxide chemoreceptors remain responsible for this response (Daxboeck and Holeton 1978, Smith and Jones 1978, Smith and Davie 1984, Sundin et al. 1999, Reid et al. 2000). It has been recognised that cardiac rate reduction under hypoxia is due to the increased activity of cholinergic fibres of the vagus nerve (Randall 1966, Randall and Smith 1967, Wood and Shelton 1980). It seems, therefore, that the bradycardia observed in our studies during Propiscin-anaesthesia of fish may have resulted from hypoxia caused by the weakening and arrest of respiratory movements.

Relationship between cardiac rate and respiration rate

The coefficients of correlation between heart rate and ventilation rate found in our studies were higher than 0.5, which indicates a connection between these functions. The lowest correlation was recorded in experiment 4, in which the fish were treated with atropine prior to the anaesthetic treatment.

An exceptionally strict correlation of both rates was found during deep anaesthesia, when the heart rate and ventilation rate were maximally slowed down. Often in such situations, each single opercular movement was accompanied by a single heart systole. A similar effect was reported by the authors who anaesthetised fish using other agents (Randall 1962, Mitsuda et al. 1982). The effect of synchronization between the heart rate and ventilation rate in dogfish "*Squalus lebruni*" (valid name *Squalus acanthias*) and gummy shark, *Mustelus antarcticus* was reported by Satchell (1960). Labat (1966) described an intensification of this correlation in carp under oxygen deficiency, while Randall and Smith (1967) found it for rainbow trout.

It can be assumed that clear synchronization of the heart rate and ventilation rate observed in our studies during anaesthesia was an expression of adjusting of gill-vessel blood flow rate to a reduced ventilation rate.

Propiscin as an anaesthetic for fish

The experiments carried out in this study allow us to conclude that Propiscin represents a very useful anaesthetic for fish. It is odourless, easily water-soluble, and causes general anaesthesia in a short time.

The 100-percent survivability of the anaesthetised carp observed in our experiments (despite keeping the fish in the anaesthetic solution much longer than beyond the recommended duration, until the stage of complete apnoea) indicates a wide margin of safety of Propiscin used in general anaesthesia of carp.

The results of our studies indicate that the anaesthetic effect of Propiscin on carp resulted from the action of etomidate on the central nervous system, the outcomes of which included reduced rate and amplitude of ventilatory movements. Bradycardia observed during the anaesthesia was probably a response to a reduced gaseous exchange in the gills.

CONCLUSIONS

- Propiscin administered at concentrations of 1 and 2 $ml \cdot l^{-1}$of water for carp results in a state of general anaesthesia accompanied by a reduced ventilation rate and bradycardia.
- Intrapericardial atropine injection prevents bradycardia in carp anaesthetised with Propiscin.
- Forced water flow through the gill system removes bradycardia in carp anaesthetised with Propiscin.
- During a Propiscin-evoked general anaesthesia in carp, there is a high correlation between heart and ventilation rates.

• Among the behavioural signals, ventilatory movements represented the most important indicator for the evaluation of Propiscin anaesthesia process in the carp.

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