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Plasma Glucose and Lactate and Hematological Changes after Handling Stresses in the Dogfish

L. Tort*, F. González-Arch and J. Balasch

Departament de Biologia Cel.lular i Fisiologia
Facultat de Ciències
Universitat Autònoma de Barcelona
08193 Bellaterra (Spain)

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Hematological variables as well as blood glucose and lactate levels are determined in the Mediterranean dogfish after either cannulation surgery or handling by capture. The results show that both types of stress generate similar metabolic changes but somehow different hematological responses, since a cell loss is detected in cannulated fish, whereas an increase of cell volume is observed after handling. At the same time, surgery stress requires longer time to recover the basal levels than handling stress.

Key words: Stress, Dogfish, Handling, Surgery.

Due to experimental requirements or surveys on fish populations, several handling procedures are currently used which can affect the basic physiological variables in fishes and induce stress responses (5, 7, 13). In the field, much of the stress situations are linked with the physical or chemical conditions of water, environmental temperature, presence of pollutants or the eventual occurrence of a predator. As far as captive populations and culture farms are concerned, the main source for stress situations is the man activity over the fish because of current farm procedures. Moreover, most of physiological studies also require a number of manipulations on individuals, from anaesthesia to surgery.

One of the common requirements for many physiological analysis is blood extraction. The caudal puncture is the most common procedure used for single extractions whereas cannulation has been used especially for chronic extractions (3, 14, 18). The first technique does not require anesthesia and involves the capture of the fish and some minutes out of water. The cannulation of the dorsal aorta of fishes is a widely used technique in fish research which allows a direct access to the blood stream (16). The cannula connected to the

* To whom all correspondence should be addressed (Tel.: 93.5811914; Fax: 93.5812003).

dorsal aorta lasts some days without major problems. Thus, chronic blood samples can be obtained or chemicals can be injected through it, thus avoiding the repetition of the stress or irreversible damage to the blood vessel. However, this technique includes surgery and anaesthesia as intrinsic procedures which may result in some degree of stress for the fish. Different authors (6, 16) recorded significant changes in hematology following cannulation procedures and important alterations on respiratory and blood gas parameters were obtained in *Scyliorhinus canicula* (4, 19).

Much work has also been done on several types of handling stress, especially in teleosts. Amongst them, the effects of capture, anaesthesia, grading, crowding and hauling have been described in teleosts (5) whereas little work is available in elasmobranchs. All these handling procedures have been shown to produce physiological changes mostly initiated by primary responses such as the release of catecholamines and corticosteroids (9). These physiological changes have been considered as secondary responses to stress resulting from the neural and hormonal primary changes induced by the stressor. The responses of the animal systems to the specific consequences (hypoxia, hyperactivity, energetic changes) of these procedures (2), should also be considered.

Hematological variables are very useful indicators to both stress and hypoxia since they provide information about the blood respiratory properties and the oxygen carrying capacity of blood. The values of glucose and lactate in blood give a complementary overview on the metabolic or energetic status. Previous work (4, 19) showed the dynamics of blood gas respiratory and hematological variables after recovery from cannulation of the dorsal aorta in dogfish. The results showed that some of these variables recovered their normal figures after 24 hours but not all of them. Thus, it was concluded that a longer period should be necessary to stabilize the physiological values of the dogfish after this kind of surgery. The present work studies the changes in hematology over a period of 48 h by comparing the two procedures for blood obtention and showing whether the fish recover after this period of time.

Materials and Methods

The species used was the dogfish *Scyliorhinus canicula* L., a common elasmobranch in Mediterranean waters. Mean fish weight was 165 g (112 to 216 g), adult size although bigger sizes are found in Atlantic waters (8). Specimen were obtained from local fishermen near Arenys de Mar (Barcelona, Spain) and transported to the Aquarium of Barcelona (Institut de Ciencies del Mar). Fishes were maintained for three months in open seawater circulation tanks (3,000 L). During this time and throughout the experiment the temperature and photoperiod followed the natural values. The feeding regime was *ad libitum* of sardines and mussels every second day.

After the stabulation time, a first group of fishes were individually taken from the big tanks and processed for cannulation surgery as described later. The second group was netted from the big tanks, held during 30 s in a small tank, taken again to perform the puncture simulation to caudal vessels and finally replaced to the big tanks. The puncture was performed without blood extraction but with all the other procedure steps. For the control group, fishes were quickly captured and blood extracted from the caudal vessels. In this case the blood extraction took less than 40 s.

In order to place the cannula, fish were previously anaesthetized by immersion for 2 min in 0.1 g/L of buffered MS222 (Sandoz) in seawater. Afterwards, fish were placed upside down on an operating table in such a way that the whole

setup allowed to maintain the mouth open in order to have access to the proximity of the dorsal aorta and so that water and anaesthetic irrigation to the gills could be supplied during the surgery. The cannula was introduced following the technique shown by SOIVIO *et al.* (16). When cannulation was successful, the cannula containing heparinized Nichols saline was sealed and the fish allowed to recover. After 3 h, 24 h and 48 h a number of 10 fishes were sampled and blood was obtained either from the cannula or from caudal puncture.

The blood was processed in order to obtain hematological and biochemical data as follows: Hematocrit and Leucocrit were determined using a microhematocrit centrifuge. Red blood cells were counted in a Neubauer chamber using Nichols saline as a dilutor. The hemoglobin concentration was obtained through a commercial kit (Boehringer-Mannheim) using a colorimetric reaction with potassium ferrocyanide. From these values, the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated (20). Blood glucose and lactate concentration were also determined using commercial kits (Boehringer-Mannheim) based on colorimetric and enzymatic methods respectively. Results were analyzed by an Analysis of Variance followed by a Duncan test to check particular differences between groups.

Results

The primary hematological measurements show some significant changes. In particular, the hematocrit values are significantly different from controls in both groups at the 3 h sample (table I). Thus, lower values are found in cannulated fishes whereas a significant increase is shown in the handled fish. Values return to normality after 24 h in both groups. Hemoglobin and red cell numbers show a similar trend, since a significant decrease at 3 h and 24 h is followed by a recovery after 48.h in cannulated fishes whereas the decrease observed in the handled group at the same periods is not significant. In terms of percentage changes it is worth noting that the biggest change in the cannulated group is the decrease in red blood cell number whereas in the handled group the greatest variation is the decrease in hematocrit.

The calculation of the combined hematological indexes are also shown in table I. As it can be seen, similar changes are detected in both cannulated and handled fishes. However, cannulated fish show longer period of changes of up to 24 h. On the other hand, the group of handled fish show a significant decrease in the hemoglobin concentration per cell whereas stable values through time are recorded in cannulated fishes.

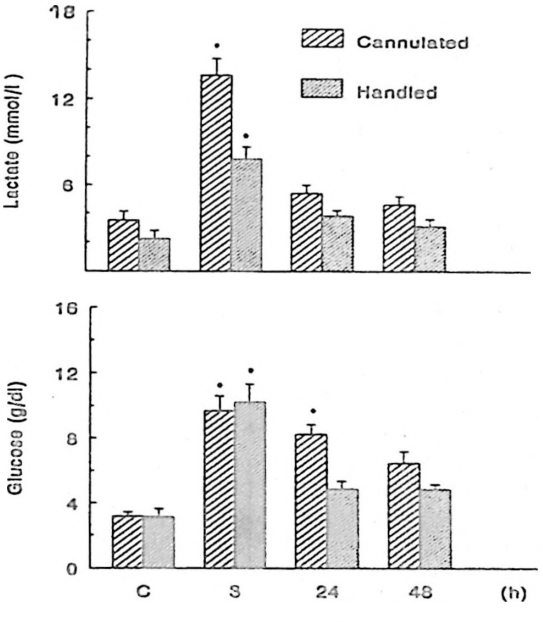

Fig. 1. *Effect of handling stresses on plasma glucose and lactate levels.*
Values are mean ± s.e.m.; n = 10 per group. *p<0.05.

Table I. *Hematological parameters analyzed in cannulated (c) and handled (h) fish.* Values are mean ± s.e.m., n = 10, per group. *p<0.05 compared with control values.

VARIABLE	GROUP	CONTROL	3 HOURS	34 HOURS	48 HOURS
Ht (%)	c	17.5 ± 0.50	15.3 ± 0.40	16.2 ± 0.40	16.4 ± 0.30
	h	18.1 ± 0.40	22.0 ± 0.50	17.5 ± 0.30	17.6 ± 0.20
Hb (mg/d)	c	4.10 ± 0.17	3.49 ± 0.12*	3.60 ± 0.14*	4.01 ± 0.13
	h	5.05 ± 0.15	3.99 ± 0.12	3.94 ± 0.15	4.06 ± 0.16
RBCC ($x10^3/mm^3$)	c	154 ± 4.32	101 ± 2.14*	123 ± 5.19*	146 ± 3.47
	h	184 ± 2.98	165 ± 6.33	162 ± 8.38	172 ± 4.26
MCV (mm^3)	c	1143 ± 37.4	1539 ± 66.2*	1343 ± 63.9*	10.95 ± 22.1
	h	985 ± 32.8	1322 ± 31.5*	1085 ± 39.2	102 ±12.7
MCH (pg)	c	268 ± 8.19	350 ± 22.10*	298 ± 12.34*	274 ± 6.26
	h	275 ± 3.38	239 ± 4.45*	245 ± 10.48	236 ± 4.48
MCHC (%)	c	23.5 ± 0.41	23.1 ± 1.52	22.4 ± 1.04	25.0 ± 0.53
	h	28.2 ± 0.77	18.1 ± 0.19*	22.6 ± 0.53	23.1 ± 0.59
Lt (%)	c	2.4 ± 0.07	2.2 ± 0.28	2.2 ± 0.15	1.9 ± 0.08
	h	2.6 ± 0.09	2.6 ± 0.19	2.5 ± 0.81	2.4 ± 0.10
Glucose (g/dl)	c	3.15 ± 0.44	15.3 ± 0.40	8.23 ± 0.62*	6.45 ± 0.73
	h	3.13 ± 0.53	10.2 ± 1.13*	4.85 ± 0.48	4.83 ± 0.43
Lactate (mmol/l)	c	3.50 ± 0.10	13.6 ± 1.13*	5.42 ± 0.31	4.63 ± 0.39
	h	2.26 ± 0.27	7,8 ± 0,86*	3.79 ± 0.40	3.11 ± 0.33

No main changes in white blood cell number are induced after handling as shown by leucocrit values. In the cannulated group no significant differences are found either although a tendency to leucopenia is found at the end of the period studied, probably caused by a decrease in lymphocyte numbers.

Plasma glucose and lactate rise significantly after both stressing procedures as shown by fig. 1. The pattern is similar in both cases, the difference being that recovery of control levels are observed in handled fishes between 24 and 48 h whereas in cannulated fishes glucose levels do not completely recover and lactate values recover at 48 hours.

Discussion

From the results it can be seen that both stress treatments induce changes in hematology, blood glucose and lactate. Thus, hyperglycemia and hyperlactemia are detected in both treatments. This is a general well known response generated by the release of corticosteroids and catecholamines to the blood stream and for rapidly supplying energy to overcome the extra needs in stress situations (9). In the present results, the glucose levels after cannulation are still higher than controls in the 24 h and 48 h groups, whereas levels are recovered at 24 h in handled fishes. A similar situation is found with blood lactate although lactate levels are recovered after 24 h in cannulated fish. This result would mean that in both experimental stress procedures, a certain degree of hypoxia or lack of oxygen occurs and therefore metabolic pathways have switched to anaerobic metabolism (1). The fact that levels recover later in cannulated fishes could be due to either a higher degree of stress or at the same time to the influence

of the anaesthetic, since some anaesthetics have been shown to induce a moderate hypoxia (17).

The same response to a situation of poor aerobic resources is detected through hematological values together with other physiological adjustments of water and ions. In reference to these variables a different pattern is observed in one experimental group compared to the other. Thus, the cannulated group shows a reduction of hematocrit, hemoglobin and red blood cell number. Therefore, a loss of blood via small hemorrhage or hemolysis could occur related to the cannulation procedure. Although no apparent hemorrhage was detected in these fishes, a small amount of blood could be lost during or immediately after the operation without a serious compromise for the fish functions. As far as the captured group is concerned, an increase of the hematocrit is recorded without concomitant rises in hemoglobin or red cell numbers. This indicates that red cells increased in volume, a process described by SOIVIO and NIKINMAA (15) as erythrocyte swelling which leads to an increase of efficiency in oxygen uptake by the hemoglobin in the red blood cells. This swelling is corroborated by the increase in the values of Mean Corpuscular Volume (table I). Thus, although this species has actually rather a higher red cell size as compared to other fishes (12), the phenomenon of swelling equally takes place. Nevertheless, a certain degree of swelling could also be identified in the cannulated group since their MCV values also increased after 3 hours although to a lesser extent.

Leucocrit values also show some changes. Although it is not a very precise indication of the white blood cell responses, it has been accepted as a general indicator of stress situation in fish (10). In the cannulated group, it can be observed that lower values are recorded in all groups when compared to controls. While in the 3 h group the values could be referred to the changes of cell volume, it should be noted that the values do not recover after 48 hours, which could indicate that a significant lymphocytopenia could possibly take place in the subsequent hours, since the decrease at 48 hours is only indicative ($p<0.1$). This lymphocytopenia would be the consequence of hormonal action on the leucocyte number through a decrease of lymphocytes as it has been shown in other works (11). In the handled group, no significant decreases are observed and therefore no significant immunodepression should be induced by primary responses with this experimental stress.

Finally, not all parameters are recovered after 24 h and even after 48 h some variables still show significant differences. These results should be taken into account in further works since the consequences of stress and the mechanisms of recovery are important enough to interfere with the measurements sought by the researchers following cannulation techniques. In conclusion, both types of stress can be said to generate responses in the dogfish and while some responses can be detected in all stresses since they are mediated by neurohormonal rapid pathways, other responses depend more on the type and intensity of the stress induced. Moreover, the introduction of anesthesia gives some advantages but at the same time produces some side effects which can interact with the physiological response to stress.

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Se determinan los niveles plasmáticos de glucosa y lactato y diversos parámetros hematológicos del pez lija del Mediterrráneo, después del estrés producido por la introducción

de una cánula aórtica o la manipulación de la captura. Los resultados muestran que los dos tipos de estrés inducen cambios metabólicos similares aunque diferentes respuestas hematológicas, ya que los peces canulados muestran pérdida de células mientras que en los capturados se registra un aumento de volumen celular. Los ejemplares canulados necesitan un mayor tiempo de recuperación.

Palabras clave: Estrés, Pez lija, Manipulación, Cirugía.

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