Nitric oxide (NO) gas present in the swim bladder of cod (Gadus morhua)

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Abstract
Nitric oxide (NO), a simple diatomic molecule, has been shown to be a biological messenger of key importance, controlling an almost limitless range of functions in the body. In the present study NO gas was measured by chemiluminescence in the swim bladder of cod staying at surface level. All swim bladders contained NO, indicating that this compound might be involved in the regulation of the volume of the swim bladder.

Key words: Cod, swim bladder, nitric oxide, nitric oxide synthase

Introduction
The swim bladder in fish is a gas-filled (mostly O₂) organ positioned at the dorsal part of the abdominal cavity. The major function of the organ is buoyancy control needed to minimize energy consumption (1). If the density of the fish was higher than the surrounding water, the fish would sink. Similarly, fish with lower density would be floating towards the surface. In both instances the fish has to use a significant amount of energy to prevent this alteration in position.

As long as the fish is not moving, this task is fairly simple. But fish move, and as soon as the fish ascends or descends, the hydrostatic pressure changes and so does the swim bladder gas volume. To compensate for this, gas must be rapidly secreted into the swim bladder while descending and removed while ascending.

Secretion of gas into the swim bladder is a result of ingenious mechanisms by which oxygen released from haemoglobin diffuses into the swim bladder (2,3). These should be rapidly reacting mechanisms with a wide gas pressure range.

Similarly, the gas-eliminating mechanisms have to be very rapid. In the main, fish can be grouped into two categories, physostomes and physoclistes (1). In the first group, the fish have a pneumatic duct from the swim bladder to the oesophagus and they can push the gas out of the mouth and fill the bladder by gulping air at the surface. Fish living deeper in the water, such as Atlantic cod, do not have the opportunity to gulp air at the surface and their swim bladder has lost its connection to the gastrointestinal tract. Smooth muscle cells in a specific region can act upon a membrane (ovalis) through which the gas can diffuse back into the bloodstream.

The mechanisms behind these very rapid gas volume changes inside the swim bladder in physoclides are not known in all details. Based upon an assumption that nitric oxide, NO, may act as a regulator in these processes we decided to investigate if NO could be found in the swim bladder of cod.

Materials and methods

Materials
Fourteen cod weighing 0.1–0.9 kg were caught with sport-fishing equipment (lures) in a fjord on the Swedish West Coast at a depth of about 10 m. The fishes were kept alive in seawater (0°C, about 60% of salinity in ocean water) at surface level and were tested within 1 h after being captured.
Experimental design

When tested, the fish was rendered unconscious by a blow to the head, the abdominal wall was opened and 3 ml of NO-free air was inserted by means of a 5 ml syringe and a thin needle into the swim bladder. After 15 s, the gas was ejected from the swim bladder and immediately injected into a rapid-response chemiluminescence analyzer (Aerocrine, Stockholm, Sweden) to determine the NO concentration (4). The instrument’s detection level for NO was 1 part per billion (ppb). Calibration of the instrument was performed with cylinder gas (10 ppm NO in nitrogen; AGA, Lidingo, Sweden).

Results and discussion

As evident from the results presented in Table I, NO gas is present in the swim bladder of cod, even when the fish has been at surface level for a couple of minutes. To the best of our knowledge, this is the first time that NO has been measured inside the swim bladder, and a key question is: does it mean anything?

NO, a simple molecule of just one atom of nitrogen and one of oxygen, was for many years looked upon as a molecule of little, if any, physiological interest (5). During the last two decades, it has been shown that this molecule is a biological messenger of key importance, controlling an almost limitless range of functions in the body. In vertebrates, including fish, NO is synthesized from the amino acid L-arginine and molecular oxygen (5,6). This reaction is catalysed by two main categories of nitric oxide synthases (NOS); constitutive (cNOS) and inducible (iNOS). In the first category, the two isoforms are identified in neuronal and endothelial cells and are named neuronal NOS (nNOS) and endothelial NOS (eNOS), respectively. It is generally accepted that the site and the onset of NO generation from constitutive NO synthase isoforms may play different roles in different physiological processes and these may act rapidly at very low molecular levels.

In contrast, iNOS requires time for up-regulation and the up-regulation may result in much higher NO levels.

Based upon this general knowledge we propose a hypothesis that the NO found in the swim bladder derives from the cNOS isoforms. So far, however, there are only two reports dealing with cNOS in the swim bladder and both reports are on physostomes. In zebrafish, it has been found that nNOS-expressing cells could be found in the mesenchyme of the swim bladder as early as only 72 h post fertilization (7). After hatching, the number of nNOS-expressing cells increased. In goldfish, it has been demonstrated that the muscular layer of the swim bladder and pneumatic duct are densely equipped with NO synthase containing neurons and fibres (8). Thus, in physostomes such as zebrafish and goldfish, NO may be able to indirectly regulate the amount of gas in the bladder. The mechanisms behind that regulation seem to be the vasodilatation of the supplying blood vessels (eNOS) and control of the blood flow, as well as more directly by controlling the smooth musculature in the bladder and the pneumatic duct (nNOS). When the fish wants to quickly release the gas in the bladder, the muscle in the swim bladder contracts to push the gas through the mouth in a reflex called the gasspuck reflex.

Physoclists, such as cod, have a closed swim bladder, thereby lacking this reflex. Instead, they have a smooth muscle in the bladder called the ovalis. The bladder of cod is covered by guanine, giving it a silvery look and preventing gas from diffusing out from the bladder. When relaxed, the ovalis exposes a membrane, not covered by guanine, through which the gas can diffuse out of the bladder. Additionally, cod have gas gland cells in swim bladder epithelium. By a mechanism called the Root effect, peculiar for fish and involved in the blood flow, O2 might diffuse into the bladder, even at a very high pressure (2,3).

If it is assumed that the cod, in order to maintain the equilibrium at a certain depth, has a constant production of gas during swimming; thereby some NO will diffuse together with oxygen into the bladder. Upon ascending to the surface, the gas in the swim bladder will expand, the receptors in the wall of the bladder would sense the volume increase, the neuronal impulses trigger NO production (probably through nNOS) followed by relaxation of the ovalis and diffusion of gas out of the bladder. If the cod descends, sensors would register the volume reduction, more NO will be produced (probably through eNOS), the blood flow will increase and more gas will enter the bladder via the Root mechanism. It should be mentioned that in table I. Nitric oxide (NO) levels (ppb) in swim bladder of cod fish.

| Cod no.  | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Weight (kg) | 0.4 | 0.4 | 0.5 | 0.4 | 0.9 | 0.3 | 0.5 | 0.2 | 0.6 | 0.5 | 0.1 | 0.3 | 0.5 |
| NO (ppb) | 70  | 80  | 90  | 75  | 60  | 75  | 65  | 39  | 50  | 40  | 60  | 130 | 90 |
physcyclists, i.e. in fish with a closed swim bladder, the swim bladder is considered to be sterile, making a microbial involvement in gas volume regulation rather unlikely.

However, whatever the mechanism(s) for the gas volume regulation in the swim bladder of cod, the mere fact that NO is present in a certain amount in the measured gas, together with the NO-producing cells and fibres presence in the wall, strongly support an assumption that NO plays a role in this regulation.

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