Cholinergic and Adrenergic Tones in the Control of Heart Rate in Teleosts. How Should They be Calculated?

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ABSTRACT. Cholinergic and adrenergic tones were calculated for three different teleost fish species: Gadus morhua, Labrus bergylta, and Sparus aurata using atropine as a muscarinic receptor antagonist and either sotalol or propranolol as β-adrenoceptor antagonists. Depending on the order of administration of atropine and the two β-adrenoceptor antagonists, it was observed that propranolol but not sotalol enhanced cholinergic tone. Thus, if propranolol is used to determine autonomic cardiac influences, it has to be injected after atropine and not before. Differences in intrinsic heart rate were observed between treatments in two of the three species studied, suggesting the activity of a non-cholinergic non-adrenergic mechanism in heart rate control in fish. Different models to calculate cholinergic and adrenergic tones are discussed. The additive model described by other authors is appropriate provided that no interaction exists between cholinergic and adrenergic influences. We demonstrate no interaction in the species studied in this experiment. Finally, a modification of the additive model that uses R-R interval instead of heart rate in the computation is proposed. This is justified with a computer simulation in terms of the linearity of the response given the reciprocal relationship between R-R interval and heart rate. COMP BIOCHEM PHYSIOL 118A;1:131–139, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Adrenergic tone, atropine, autonomic interaction, cholinergic tone, heart rate control, propranolol, sotalol, teleost

INTRODUCTION

The autonomic nervous system is the main short-term modulator of heart rate in vertebrates. A double antagonistic innervation has been demonstrated in all vertebrate groups with exception of cyclostomes, elasmobranchs, dipnoans and some groups of teleosts. Even in species lacking a direct spinal autonomic innervation of the heart the circulating catecholamines released from the chromaffin tissue will have a positive chronotropic and inotropic effect on the heart (7,17).

The role of vagal efferents in inducing heart rate (HR) changes was first demonstrated using electrical stimulation (13,18,36). More contemporary studies have used specific agonists/antagonists to further study the double control of the heart (8,11). Muscarinic receptor antagonists induce a rise in heart rate by blocking cholinergic post-synaptic receptors and reducing the inhibitory parasympathetic drive. On the other hand, β-adrenoceptor antagonists antagonize competitively the natural catecholamines at the level of cardiac β-receptors, which results in a negative chronotropic and inotropic effect.

Several models of autonomic chronotropic cardiac control have been described. Although these models were based on experiments using electrical stimulation in mammalian hearts (35,37), they have been widely employed to characterize cholinergic and adrenergic tonus on the heart in most vertebrate groups including teleosts (2,8,11). They adopted the mathematical formulation reported by Lin and Horvath (29), which is based in the additive model of Rosenblueth and Simeone (35).

In the light of new findings in relation with autonomic cardiac control in mammals, it seemed important to re-evaluate and adapt the methods to attain an accurate quantification of cholinergic and adrenergic tones. First question is, how should the cholinergic and adrenergic tones be calculated? Second, and most important, the additive model of Rosenblueth and Simeone (35) does not account for possible interactions between the cholinergic and adrenergic autonomic influences. The existence of such interaction in resting conditions has been termed “accentuated antagonism” (28) and is still controversial in mammals (27). To our knowledge, it has not been described in teleosts.

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This topic can be approached pharmacologically by the injection of autonomic antagonists in different order. Axelsson et al. (5) used this protocol in Myoxocephalus scorpius and did not find differences. If interaction exists one would expect that, for example, after the administration of a β-adrenoceptor antagonist, there would be a compensatory response of the parasympathetic branch in order to minimize the effect of the reduced adrenergic influence on the heart. Such “compensatory demand” has been observed in mangrove dogs in exercise conditions (9).

MATERIALS AND METHODS
Species and Handling Procedures
The study involved several experiments in different teleost species: 11 individuals of the Atlantic cod Gadus morhua (558 ± 174 g), 10 individuals of the ballan wrasse Labrus bergylta (172 ± 17 g), and 22 individuals of the Mediterranean sea bream Sparus aurata (210 ± 30 g) were used in the study. The animals were kept in well aerated tanks for at least 1 week before any surgical procedure took place.

G. morhua were maintained at 10°C, L. bergylta at 20°C (the animals were acclimated at that temperature for at least 1 week) and S. aurata at 16°C.

For comparison, results from a previously published study in the bull-rout M. scorpius (5) will also be used in the present work. In this early study, the same experimental protocol described here was employed (see below).

Two different anesthetics were used. G. morhua was anaesthetized with MS222 (0.1 g·L⁻¹, SIGMA). 2-Phenoxyethanol (0.75 mL·L⁻¹, SIGMA) was used for S. aurata and L. bergylta. The anesthetic dose was chosen in order to get a similar induction time (2 min) for both agents. Once anaesthetized, the fish was weighed to the nearest gram and placed ventral side up on an operation table, and the gills continuously irrigated during the entire surgery.

Heart Rate Measurements
Heart rate was obtained from the electrocardiographic signal. Two enamel-coated stainless steel wires (0.2 mm in diameter, supplied by Driver Harris S.A. and insulated by Asmalibar S.A.) were attached to the fish forming a bipolar lead. Both electrodes were anchored to the skeleton surrounding the pericardial cavity: a first electrode midventrally where both cleithra bones join and a second electrode between the fused structures of the pelvic girdle [see (19) for a general anatomical description]. The depth at which each electrode was inserted was established after anatomical dissection of the different species. Both wires were sutured to the skin in two places: ventrally between the pelvic fins and dorsally at the base of the dorsal fin, preventing relative displacements of the electrodes and improving the signal quality. The wire electrodes were passed through a stainless steel tube and soldered to an appropriate connector placed on the lid of the chamber in which the fish was maintained. With this setup, the stainless steel tube worked as the reference electrode being in direct contact with the water, and minimum interferences were introduced in the signal.

The ECG signal was amplified and filtered in a Grass Polygraph Recorder System (Model 7D) and digitalized in a PC computer equipped with a LabPC+ card and LabView software (©National Instruments Co.). Once digitalized, the QRS complex for each beat was detected in real time and instantaneous heart rate stored.

Pharmacological Administration and Experimental Protocol
For the drug injections, the ventral aorta was chronically cannulated using a PE-50 tubing via the afferent branchial artery of the third gill arch (5). The cannula was filled with heparinized saline (100 I.U.·mL⁻¹). The cannula was secured with two skin sutures. The surgery was performed in less than 20 min and the animals were returned to the experimental chamber, where they were allowed to recover for at least 24 hr.

After the recovery period, heart rate was recorded for 1 hr before any manipulation of the animals took place. After this initial recording period, the animals received a first injection of either a muscarinic receptor antagonist (Atropine sulfate, 1.2 mg·kg⁻¹, SIGMA) or a β-adrenergic antagonist (S-propranolol hydrochloride, 3.0 mg·kg⁻¹, FLUKA in L. bergylta and S. aurata or Sotalol hydrochloride, 2.7 mg·kg⁻¹, Bristol Myers Squibb in G. morhua and S. aurata). Thirty min after this injection the ECG was recorded for another hour. Once the effects of the first injection were recorded, the second antagonist (depending on which one was given first) was injected and similarly, a 1 hr recording was taken 30 min later (see Fig. 1 for details).

The same convention used in a previous paper (1) will be employed to refer to the different treatments: GI for those animals receiving the β-adrenergic antagonist (sotalol or...
propranolol) before atropine and GII for those receiving atropine first.

**Calculation of Cholinergic and Adrenergic Tones**

We propose the use of the following equations for the quantification of cholinergic and adrenergic tones:

**GI (Sotalol first)**

\[
\text{Chol}(\%) = \frac{(R-R)_β - (R-R)_0}{(R-R)_0} \times 100
\]

\[
\text{Adr}(\%) = \frac{(R-R)_β - (R-R)_\text{cont}}{(R-R)_0} \times 100
\]

**GII (Atropine first)**

\[
\text{Chol}(\%) = \frac{(R-R)_\text{cont} - (R-R)_\text{musc}}{(R-R)_0} \times 100
\]

\[
\text{Adr}(\%) = \frac{(R-R)_0 - (R-R)_\text{musc}}{(R-R)_0} \times 100
\]

where:

- \((R-R)_\text{cont}\) — Control R-R interval.
- \((R-R)_β\) — R-R interval after β-adrenoceptor blockade.
- \((R-R)_\text{musc}\) — R-R interval after muscarinic receptor blockade.
- \((R-R)_0\) — R-R interval after complete autonomic blockade.
- Chol(%) — Cholinergic tone in percent.
- Adr(%) — Adrenergic tone in percent.

The rationale to use these equations will be discussed in another section.

**Statistics**

The non-parametric Mann-Whitney test was used to assess differences in intrinsic HR between GI and GII groups for each species. An ANCOVA design with previous logarithmic transformation of the data was employed to test the differences in cholinergic and adrenergic tone between GI and GII using control HR as a covariant. All tests were applied using SPSS software.

**RESULTS**

Heart rates and cholinergic and adrenergic tones in the different species depending on the injection order are shown in Table 1. This data set was statistically analyzed using an ANCOVA design in which control R-R interval was used as a covariant. This design was preferred because it allows the decomposition of intra-group variance in real inter-animal variance and variance due to a different initial vagal drive. In other words, part of the intra-group variance is reflecting the linear relationship between vagal drive and control R-R interval (see Fig. 2) and can be dismissed from the analysis using the covariant. No correlation was found between control R-R interval and adrenergic tone.

The ANCOVA revealed differences \((p < 0.05)\) between GI and GII in those experimental groups in which propranolol was used as a β-antagonist, with the exception of the adrenergic tone for *L. bergylta*. No differences between GI and GII were found when sotalol was used as a β-antagonist. A comparison between propranolol and sotalol treatments is shown for *S. aurata* in Fig. 3.

Finally, the intrinsic R-R interval \((R-R)_0\), i.e., the R-R interval after complete blockade with atropine and sotalol/propranolol, differs between GI and GII in three of the four species studied: *L. bergylta*, *M. scorpius*, and *S. aurata* (Fig. 4). When \((R-R)_0\) differs between treatments, the lowest HR, appears when atropine is injected first in those species in which cholinergic tone is more important than adrenergic tone (*L. bergylta* and *S. aurata*) and the opposite is true for *M. scorpius*, in which adrenergic tone is quantitatively more important than cholinergic tone.

**DISCUSSION**

**Critique of Method**

Atropine at the dose employed (1.2 mg · kg\(^{-1}\)) has a fast antagonistic action on muscarinic receptors. In contrast with the mild hypertension reported in humans (24,27), atropine does not induce changes in blood pressure in the species studied ([5,8]; Altimiras, unpublished observations). Being so, there is no need to compensate for reflex changes in heart rate associated with hypertensive effects (24). The effects are also long lasting, and preliminary studies showed no substantial recovery in vagal tone after 24 hr in *S. aurata* or *L. bergylta*.

Beta-adrenergic antagonists are known to be less specific and this needs to be taken into account when used to analyze adrenergic cardiac tone. Propranolol, for instance, is a lipophilic molecule with membrane stabilizing properties that also acts as a local anesthetic, independent of its effects as a β-blocker (30). Aside from that, it enhances vagal tone in humans (27) and the same effect has been observed for atenolol (14) and sotalol (21). From this study, it appears that propranolol but not sotalol facilitated vagal drive at the doses employed in *S. aurata*, and this facilitation explains the differences in tones between GI and GII when propranolol was used (Table 2). When propranolol is injected first (GI) it not only abolishes adrenergic tone but it also enhances vagal drive, as can be seen by the larger change in R-R interval induced by atropine when injected after propranolol in comparison when injected before (see Fig. 3). Interestingly, the same difference between GI and GII treatments using propranolol was found in *L. bergylta*.

We also observed that the effects of β-antagonists disappeared more rapidly than the effects of atropine. At the
TABLE 1. Heart rates before and after autonomic blockade

<table>
<thead>
<tr>
<th></th>
<th>HR_cont</th>
<th>HR_atro</th>
<th>HR_b</th>
<th>HR_g</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GI</td>
<td>GII</td>
<td>GI</td>
<td>GII</td>
</tr>
<tr>
<td>G. morhua</td>
<td>40.0 ± 3.7</td>
<td>37.2 ± 2.4</td>
<td>45.8 ± 1.3</td>
<td>33.5 ± 2.2</td>
</tr>
<tr>
<td>L. bergylta</td>
<td>90.4 ± 9.2</td>
<td>84.5 ± 8.6</td>
<td>111.6 ± 3</td>
<td>72.9 ± 7.2</td>
</tr>
<tr>
<td>S. aurata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sotalol</td>
<td>66.4 ± 4.4</td>
<td>63.4 ± 5.4</td>
<td>89.0 ± 2.2</td>
<td>60.4 ± 4.0</td>
</tr>
<tr>
<td>Propranolol</td>
<td>57.5 ± 2.5</td>
<td>* 66.1 ± 2.2</td>
<td>83.3 ± 3.9</td>
<td>43.9 ± 2.2</td>
</tr>
<tr>
<td>M. scorpius</td>
<td>41.8 ± 1.4</td>
<td>* 48.3 ± 2</td>
<td>53.0 ± 1.5</td>
<td>32.3 ± 1.1</td>
</tr>
</tbody>
</table>

Data (%) are expressed as Mean ± SEM.

*Indicates differences between treatments ($p < 0.05$).

doses used, however, the effects on heart rate were consistently maintained for at least 5 hr after injection in preliminary studies.

Given the observed secondary effects of propranolol, especially its vagal accentuation, we suggest sotalol to be a better $\beta$-adrenoceptor antagonist than propranolol, although the amiodarone-like activity of sotalol as an antiarhythmic may also have undesirable interfering effects, not yet described.

Finally, it is important to emphasize that adrenergic and not sympathetic tones are obtained with the protocol used.

Sympathetic tone refers to the tonic activity of neural sympathetic innervation and, as such, does not include the effect of circulating catecholamines. Bretylium, a drug that prevents the release of catecholamines from post-synaptic adrenergic neurons, can be used to assess the adrenergic nervous tone as opposed to the total adrenergic tone (2). However, the severe and long lasting side effects of bretylium and its difficult application in fish has prevented its exten-

FIG. 2. Relationship between control R-R interval (of each individual animal) and cholinergic tone (squares) and adrenergic tone (triangles) in Labrus bergylta. Notice the clear increase in cholinergic tone when R-R intervals are longer (and heart rates are reciprocally smaller). Each data point corresponds to a single animal.

FIG. 3. R-R intervals in Sparus aurata in control conditions and after partial and complete autonomic blockade. The drugs employed in each case are specified: Atr—atropine, Sot—sotalol, Pro—propranolol. Data plotted as Mean ± SEM. GI treatment indicated as closed symbols, GII indicated as open symbols.

FIG. 4. Intrinsic heart rate after complete pharmacologic blockade in the different species using sotalol (top panel) or propranolol (bottom panel) as a $\beta$-adrenoceptor antagonist. Data from Myoxocephalus scorpius come from a previous study (5). GI indicated as closed symbols, GII indicated as open symbols. *—significant differences between treatments at $p < 0.05$. 

FIG. 5. Relationship between control R-R interval (of each individual animal) and cholinergic tone (squares) and adrenergic tone (triangles) in Myoxocephalus scorpius. Notice the clear increase in cholinergic tone when R-R intervals are longer (and heart rates are reciprocally smaller). Each data point corresponds to a single animal.
TABLE 2. Cholinergic and adrenergic tones in the different species in both treatments GI and GII

<table>
<thead>
<tr>
<th>Species</th>
<th>GI (%)</th>
<th>GII (%)</th>
<th>GI (%)</th>
<th>GII (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. morhua</td>
<td>15.1 ± 2.9</td>
<td>21.3 ± 5.1</td>
<td>17.3 ± 3.9</td>
<td>12.9 ± 1.4</td>
</tr>
<tr>
<td>L. bergylta</td>
<td>50.7 ± 11.6</td>
<td>30.4 ± 11.5</td>
<td>28.4 ± 4.9</td>
<td>20.8 ± 2.9</td>
</tr>
<tr>
<td>S. aurata</td>
<td>49.7 ± 8.2</td>
<td>38.8 ± 14.8</td>
<td>12.7 ± 2.4</td>
<td>16.8 ± 4.8</td>
</tr>
<tr>
<td>Sotalol</td>
<td>84.1 ± 13.7</td>
<td>22.4 ± 1.8</td>
<td>44.1 ± 5.2</td>
<td>12.8 ± 3.3</td>
</tr>
<tr>
<td>Propranolol</td>
<td>13.2 ± 3.3</td>
<td>8.2 ± 1.7</td>
<td>25.5 ± 3.8</td>
<td>20.2 ± 0.9</td>
</tr>
</tbody>
</table>

Data (%) are expressed as Mean ± SEM.
*Indicates differences between treatments (p < 0.05).

ductive use and β-adrenoceptor antagonists are still used as the most common method to characterize the adrenergic tone of the heart.

A second critique that could be raised lies on experimental design itself. A random administration of both treatments (GI and GII) in the same individuals in successive days would have been the optimal experimental choice. The long lasting effects of atropine, the unknown wash-out time of the β-blockers and the difficulty of keeping cannulae working for long-enough periods of time, prevented such design.

Calculation of Tones

The previous literature in fish (2,5,11) consistently used the equations given by Lin and Horvath (29) to calculate cholinergic and adrenergic tones (2). The equations we used, as described in the Materials and Methods section, substitute HR values for R-R intervals.

In order to analyze in detail the differences between tones calculated with either formulation, we simulated different cholinergic and adrenergic influences at different control HR using both treatments (GI and GII) in a computer model. The output of this model for a GII treatment is shown in Fig. 5A and B. We obtained two important conclusions:

1. Because tones are calculated as % changes, both calculations are independent of control HR, i.e., for a given change in HR, no matter what the control HR was, we obtain the same tonic. This property indicates that both formulations can be used to compare different species regardless of their control HR.

2. The two methods do not provide similar values at identical conditions, i.e., it is not the same to calculate tones using HR as using R-R interval. This can be seen in Fig. 5A and B. Each plot shows cholinergic (X-axis) and adrenergic tones (Y-axis) calculated from R-R values versus cholinergic tone or adrenergic tone (Fig. 5A and B, respectively) calculated from HR values. It is clear that the two methods provide different results and only when these tones are smaller than 20%, a close agreement is obtained, as shown by the black areas in Fig. 5A and B.

Katona et al. (24) compared resting sympathetic and parasympathetic influences on sinoatrial function in athletes and non-athletes quantifying HR changes after atropine or propranolol injections. Their results confirmed the “accentuated antagonism” phenomenon previously described on electrical stimulation preparations. However, “accentuated antagonism” has been refuted by a more recent study that used a similar protocol but analyzed R-R interval data rather than HR (27). Kollai et al. recalculated the data obtained by Katona et al. in terms of R-R interval, and showed that there was no evidence for any “accentuated antagonism” when using R-R intervals instead of HR.

Despite these differences, the question of which is the best model to use still remains. The rationale to use R-R interval based calculations is that neural frequency discharge rates have been shown to be linearly related with the beat-to-beat interval (26), and consequently it will be reciprocally related with HR given the reciprocal relation between R-R interval and HR (HR(Hz) = 1/R-R(sec), see Fig. 5C). Although these measurements were made in a mammalian species, we think that it is fair to extend this relationship to all vertebrate species. In conclusion, the R-R interval-based method to quantify cholinergic and adrenergic tones in teleosts (and in other vertebrate groups) offers a better estimate of these tones because it behaves linearly in relation with the changes in R-R interval that are, in turn, linearly related with nerve discharge frequency.

To further corroborate this conclusion we used our model to calculate the cholinergic and the adrenergic tone in a GII treatment if, at a given R-R interval (2 sec, equivalent to a HR = 30 bpm), the antagonists induced progressive changes identical in magnitude but in opposite direction on the R-R interval. The output for cholinergic tone is given in Fig. 5D (it is identical for adrenergic tone because equal influences were employed in the simulation). From the figure it is again clear that variations in R-R interval are linearly related with cholinergic tone when the R-R interval-based model was used. Cholinergic tones obtained from the
FIG. 5. A and B) Cholinergic (X-axis) and adrenergic tones (Y-axis) calculated from R-R values versus cholinergic (A) and adrenergic tones (B) calculated from HR values. The shaded areas in each plot indicate a close agreement (5%) between the tones calculated using HR and R-R values. C) Graphical plot of the reciprocal relationship between HR and R-R interval. D) Effect of the use of different models (R-R based or HR-based models) on the calculation of cholinergic tone with stepwise changes in R-R interval after muscarine receptor blockade. See text for more details.

HR-based model were not linear and consistently overestimated cholinergic tone when this was larger than 20%.

A survey of the fish literature provided cholinergic and adrenergic tones in 15 species, being all the measurements based on the HR based model and under a GII protocol. Table 3 provides a recalculation of these tones using our R-R based model. As described above (see also Fig. 5A and B), there is a close agreement between both models for those species with low tones such as Gadus morhua, Labrus myxus, Labrus bergylta and Myxocephalus scorpius. However, large differences are found for those species with large tones such as the antarctic fishes Pagothenia bernacchii and Pagothenia borchgrevinkii and the goldfish Carassius auratus.

**Interactions in the Autonomic Nervous System**

As previously mentioned, both formulations discussed above are based on an additive model of autonomic heart rate control (35,37) that assumes no autonomic interaction. The simplest formulation of this model is the one given by Rosenblueth and Simeone (23):

\[
\text{HR}_c = c * s * \text{HR}_0
\]

where \(\text{HR}_c\) is the heart rate, \(\text{HR}_0\) the intrinsic heart rate and \(c\) and \(s\) the factors by which cholinergic and adrenergic factors modify \(\text{HR}_0\), respectively.

In order to introduce an interaction between both, Cavero et al. (12) introduced a third multiplicative factor (i):

\[
\text{HR}_c = c * s * \text{HR}_0 * i
\]

Interestingly, when Cavero et al. employed this second model to their pharmacological data, they observed that the interaction factor was not significantly different from 1 (\(i = 0.985 \ p > 0.05\)), suggesting that there was no interaction between the cholinergic and adrenergic influences.

For the G. morhua data set we obtained an interaction factor of 0.988, again suggesting no interaction between adrenergic and cholinergic outflows. Unfortunately, differences in intrinsic R-R interval between GI and GII treatments precluded the calculation of the interaction factor in the other data sets.

Thus, it is concluded that an additive model of auto-
TABLE 3. Survey of mean cholinergic and adrenergic tones for different fish species and recalculation of tones based on the R-R model proposed in this paper

<table>
<thead>
<tr>
<th>Species</th>
<th>T (°C)</th>
<th>Chol %</th>
<th>Adr %</th>
<th>Chol %</th>
<th>Adr %</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>Oncorhynchus kisutch</td>
<td>11–13</td>
<td>55.2</td>
<td>60.0</td>
<td>34</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td>Gadus morhua</td>
<td>10–11</td>
<td>37.7</td>
<td>21.0</td>
<td>39</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Hemitrupetanus americanus</td>
<td>10–12</td>
<td>34.8</td>
<td>39.6</td>
<td>23</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>Pagothenia borchgrevinki</td>
<td>0–0.5</td>
<td>55.4</td>
<td>3.2</td>
<td>112</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Pagothenia bernacchi</td>
<td>0–0.5</td>
<td>80.4</td>
<td>27.5</td>
<td>130</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Carassius auratus</td>
<td>20–25</td>
<td>66.0</td>
<td>22.0</td>
<td>97</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Pollachius pollachius</td>
<td>11–12</td>
<td>19.7</td>
<td>33.2</td>
<td>13</td>
<td>25</td>
<td>5</td>
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<td>Labrus mixtus</td>
<td>11–12</td>
<td>14.2</td>
<td>14.7</td>
<td>12</td>
<td>14</td>
<td>5</td>
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<tr>
<td>Labrus bergyla</td>
<td>11–12</td>
<td>33.9</td>
<td>15.4</td>
<td>36</td>
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<td>Ciliata mustela</td>
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<td>14.5</td>
<td>29.6</td>
<td>9</td>
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<td>5</td>
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<td>Raniceps raninus</td>
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<td>12.5</td>
<td>28.8</td>
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<td>Zoarces viviparus</td>
<td>11–12</td>
<td>18.9</td>
<td>67.1</td>
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<td>5</td>
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<td>Myxocephalus scorpius</td>
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<td>11.5</td>
<td>25.6</td>
<td>8</td>
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<td>5</td>
</tr>
<tr>
<td>Thunnus thynnus</td>
<td>24–26</td>
<td>—</td>
<td>—</td>
<td>58.1</td>
<td>4.1</td>
<td>25</td>
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<tr>
<td>Katsuwonus pelamis</td>
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<td>—</td>
<td>—</td>
<td>130.8</td>
<td>5.8</td>
<td>25</td>
</tr>
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nomic cardiac control is appropriate to explain the adrenergic and cholinergic influences on heart rate in teleosts in basal conditions.

However, autonomic interaction may still occur in other conditions. In mammals neuropeptide Y is co-released from post-synaptic noradrenergic fibers during exercise (32) and attenuates vagal drive via inhibition of acetylcholine release from preganglionic vagal neurones. In the dogfish, Squalus acanthias, Xiang et al. (38) proposed an enhanced contractility mediated by neuropeptide Y due to a preferential stimulation of α-adrenoceptors on the heart. Neuropeptide Y has also been found in the heart of two species of skates, Raja erinacea and Raja radiata (10), but the functional significance is unclear. There is also one report of P1, purinoceptors in the heart of the dogfish mediating a negative inotropic and chronotropic effects in the atrium (31). VIP has been shown to be co-localized with acetylcholine in post-ganglionic parasympathetic neurons in dogs and rats (16) and is responsible for a vagally induced tachycardia (20). Intraarterially injected VIP produce an increase in the stroke volume of the heart in the Atlantic cod, G. morhua, but again the exact mechanism is not known (22).

Non-adrenergic Non-cholinergic Control and Intrinsic Heart Rate

It is commonly accepted that neural autonomic influences and circulating catecholamines are the main effectors on the intrinsic pacemaker rhythm of the heart although there are other mechanisms like the stretch of the pacemaker cells and non-catecholamine hormonal effects that could also play a role (15). According to this statement, the quantification of the cholinergic and the adrenergic tones of the heart would explain most of the extrinsic effectors of cardiac rhythm.

However, we observed a clear difference in intrinsic heart rate between treatments in most of the species studied in these experiments with the exception of G. morhua (Fig. 4). This result was completely unexpected because the only difference between both groups was the order in which the drugs were injected.

This observation could be related with the “excess tachycardia” reported in dogs (34) that is due to the positive chronotropic effect of VIP (33). In fact, bilaterally vagotomized individuals of S. aurata have an intrinsic heart rate of 62.0 ± 2.7 bpm (Aissaoui, unpublished results), much lower than the intrinsic rate with any of the pharmacological treatments reported here (83.2 ± 2.2 and 79.9 ± 4.6 bpm in GI, 79.9 ± 4.6 and 72.2 ± 1.5 bpm in GII, with the sotalol and propranolol treatments, respectively).

The conflict of not knowing which is the actual intrinsic heart rate at a given temperature does not compromise the methodology proposed in this paper for assessing cholinergic and adrenergic tones but raises a concern on how important other effector mechanisms can be in determining the heart rate of an animal at some given conditions.

We speculate that non-adrenergic/non-cholinergic (NANC) effectors will be more important when the animals face environmental challenges (e.g., temperature, exercise, hypoxia). In this study, L. bergyla acclimated at 20°C (above the optimal temperature for the species), showed the largest difference in HR0 between treatments (106.7 ± 4.1 bpm in GI and 88.3 ± 3.4 bpm in GII). Furthermore, in Pollachius pollachius there is an exercise-induced tachycardia not eliminated by atropine and sotalol in (5).

In summary, the results reported here could support the existence of a NANC effector mechanism although further work measuring stroke volume simultaneously with heart rate would be required in order to exclude intrinsic mecha-
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