Cardioactive effects of diphenhydramine and curcumin in *Daphnia magna*

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Although used as a model for examining the cardioactive effects of various compounds, the neuromuscular regulation of the heart of the crustacean *Daphnia magna (D. magna)* is not well understood. In the present study, we sought to determine how the heart rate of *D. magna* was affected by two previously untested compounds: curcumin and diphenhydramine (DPHM). DPHM produces a number of cardiotoxic side effects in vertebrates, particularly sinus tachycardia. Curcumin acts as a monoamine oxidase inhibitor (MAOI) and was expected to increase the heart rate of *D. magna*. DPHM was found unexpectedly to lower the heart rate of *D. magna* with time. Curcumin increased heart rate when administered in higher concentrations. However, co-administration of curcumin with DPHM negated this effect. These findings may be explained by the potential role of histamine as a sympathetic cardiac neurotransmitter in *D. magna*.

Key words: curcumin; histamine; DPHM; heart rate; H1-histamine receptor; *D. magna*

Introduction

Daphnia magna, commonly known as water fleas, have been frequently used as a model organism for testing cardioactive compounds. These planktonic crustaceans are translucent, easily maintained, and quickly affected by compounds introduced into their surrounding environment (Campbell et al., 2004). Additionally, *D. magna* and humans show similar cardiac response to various drugs (Villegas-Navarro et al., 2003; Postmes et al., 1989). Unlike higher order species of *Crustacea, D. magna*, have a myogenic heart with a cardiac pacemaker similar to the hearts of vertebrates (Spicer, 2001). This heart is composed of a thin layer of myocardial cells, making it easily modulated by small concentrations of cardioactive compounds (Stein *et al*., 1966). Thus, the physiology of the *D. magna* heart makes it a particularly useful model.

Prior studies have shown that parasympathetic regulation of the *D. magna* heart is similar to that of vertebrates. Acetylcholine had a negative chronotropic effect on *D. magna,* while atropine, an antagonist of the muscarinic M2 acetylcholine receptor, had a positive effect (Baylor, 1942; Becker and Krijgsman, 1951, see Villegas-Navarro et al., 2003). These findings match studies of the pharmacology of the vertebrate heart (Blumenthal et al., 1968). However, sympathetic stimulation of the heart of *D. magn*a appears to differ from the myogenic vertebrate heart. Epinephrine and other sympathomimetic ß-adrenergic agonists have showed both weak positive and negative chronotropic effects (Villegas-Navarro et al., 2003; Postmes et al., 1989). Additionally, several ß-adrenergic antagonists have been shown both to increase and decrease the heart rate of *D. magna* in different studies (Villegas-Navarro et al., 2003; Dzialowski et al., 2005). It is possible that these seemingly contradictory results may stem from differences in concentration and exposure time. However, this ambiguity indicates possible differences between sympathetic regulation of *D. magna* and vertebrate hearts. Thus, it is

necessary first to clarify the neuromuscular interactions of the myogenic *D. magna* heart to establish the utility of this animal as a model organism.

In the present study, the cardioactive effects of diphenhydramine (DPHM) and curcumin (diferuloylmethane) in *D. magna* were tested. These drugs have not been tested in *D. magna* prior to this study. The effects of these drugs on the heart rate of *D. magna* were assessed to provide a better understanding of the sympathetic and parasympathetic regulation of the *D. magna* heart.

DPHM is an ethanolamine derivative and first generation H1-antihistamine. It is among the oldest H1-antihistaminergic drugs and is commonly used in anti-allergy medications. H1-histamine receptors are G-protein-coupled receptors that activate the phospholipase C and the phosphatidylinositol (PIP2) signaling pathways. These receptors are found in smooth muscles, vascular endothelial cells, heart tissues, and the central nervous system in vertebrates (Reiner and Kamondi, 1994; Mitsuchashi and Payan, 1989). Activation of H1 receptors on smooth muscle cells has been shown to generate a rise of intracellular Ca^{2+}, which in turn enhanced Ca^{2+} activated potassium channel activity and served to hyperpolarize cells (Ishikawa et al., 1993). As an inverse agonist of the H1-histamine receptor, DPHM binds and stabilizes the inactive conformation of the H1-histamine receptor. This prevents the transduction of the H1-receptor signaling pathway and diminishes potassium channel activity, resulting in a depolarizing effect (Leurs et al., 2002).

First generation H1-antihistamines are known to have a number of adverse side-effects, likely due to a relative lack of selectivity for the H1-receptor and other bioactive roles (Clark and Vance, 1992; Koppel et al., 1987). In addition to relieving allergy symptoms, DPHM can act as a serotonin reuptake inhibitor (SSRI) and as a highly-potent inhibitor of the muscarinic M2 acetylcholine receptor (Simons, 2004; Liu et. al., 2006), potentially leading to cardiac toxicity (Fahy et al. 1989; Kirk, 1998; Richmond and Seger, 1985). DPHM overdose has been associated with sinus tachycardia, ventricular tachycardia, ventrical arrhythmias, *torsades de points*, cardiogenic shock, and hypertension (Yap and Camm, 2002; de Abajo and Rodriguez, 2000). Additionally, DPHM and other ethanolamine derivatives have been linked to somnolence, lethargy, and increases in electroencephalographic abnormalities (Emadian et al., 1996; Litovitz et al., 2000). In rare cases, DPHM overdose has produced delirium and psychosis (Jones et al., 1986; Serio, 2004).

While DPHM produces an elevated heart rate in humans and other mammals, its effects in crustaceans have not been studied. However, given that DPHM acts as a muscarinic M2 acetylcholine receptor antagonist, it was expected to have an atropine-like negative chronotropic effect in *D. magna* (Becker and Krijgsman, 1951; see Villegas-Navarro et al., 2003). Thus, the chronotropic effect of DPHM in *D. magna* was determined to compare the cardiac pharmacology of *D. magna* to mammalian models.

Curcumin is a compound with a number of bioactive roles and a rich history. Briefly, curcumin is the principle bioactive agent of the common spice known as turmeric. In addition to its culinary purposes, turmeric is frequently used in South Asian and Chinese holistic medicine as an anti-inflammatory agent (Goel et al., 2008). In addition to curcumin, turmeric contains two other structurally and pharmacologically similar curcuminoids: curcumin II (demethoxycurcumin) and curcumin III (bisdemethoxycurcumin) in smaller quantities (Almeida et al., 2005; Naito et al., 2002).

Curcumin has recently gained interest for its plethora of potential medicinal uses. Numerous *in vivo* and *in vitro* studies have shown that curcumin acts as an anti-oxidant, anti-carcinogen, anti-depressant, and iron chelator (Weber et al., 2005; Kunnumakara et al., 2008; Xu et al., 2005; Jiao et al., 2006). Curcumin has no known toxic effects in humans or other animals and is safe in even high doses (Lao et al., 2006). Despite these findings, curcumin has an extremely low bioavailability. This is likely due to its hydrophobic character, as well as the glucuronidation and sulfation of curcumin and curcumin metabolites in plasma (Anand et al., 2007). However, these metabolites maintain some of curcumin's bioactive

properties and may therefore contribute to its potency (Naito et al., 2002).

The cardioactive effects of curcumin have not been examined previously. Yet, there are a number of reasons to suspect that curcumin could have an effect on the heart rate of *D. magna*. Curcumin is known to act as an inhibitor of MAOA and B (Xu et al., 2005; Kulkarni et al., 2008). MAOIs have been found to increase heart rate (Dawson, 1995), though the cause of this side-effect is not well understood. Combination of MAOIs and SSRIs can produce tachycardia, believed to result from excessive serotonin (Boyer and Shannon, 2005). However, it is possible that the mechanism could be more direct. Both MAO-A and MAO-B have been identified in mammalian cardiac tissue (Rodriguez et al., 2001). Additionally, Crout et al. (1960) showed that administering MAOIs to rats could increase norepinephrine levels in heart tissue. While this would be expected to increase heart rate, the cardiac effects of MAOI-induced enhanced norepinephrine levels have not been studied. However, norepinephrine transport inhibitors are known to increase heart rate (Strempel et al., 2008). Thus, blocking norepinephrine degradation would very likely have a positive chronotropic effect. Curcumin could therefore increase heart rate by preventing the degradation of norepinephrine.

Curcumin has been shown to localize to cardiac tissue, though no effects on heart rate have been reported. Ryu et al. (2006) found that cardiac curcumin levels in mice peaked two minutes after curcumin administration and quickly decreased within 30 minutes. This short retention period could explain why curcumin has not been found to have a chronotropic effect in vertebrates. Yet it is very possible that an effect might be seen in the more ductile heart of *D. magna* (Stein et al., 1966).

The responses of *D. magna* to curcumin and DPHM were studied to elucidate differences and similarities in the pharmacology of the heart of *D. magna* and vertebrates. Unpurified, over the counter products were used as sources for both compounds. Thus, the results of the present study were confounded by a number of potential factors. Compounds were tested both alone and in conjunction with different concentrations of curcumin. DPHM was expected to elevate the heart rate of *D. magna* by directly antagonizing the muscarinic M2 acetylcholine receptor. Curcumin also was expected to raise heart rate by increasing cardiac norepinephrine levels. Co-administration of curcumin with DPHM was expected to increase the heart rate of *D. magna* further.

Materials and Methods

Animals

D. magna were ordered from Ward's Natural Science (Rochester, NY). They were stored in loosely lidded glass jars filled with spring water and kept continuously under a portable laboratory lamp issue No. G-998 (Underwriters Laboratories, Camas, WA) to maintain a high rate of activity (Stearns, 1975). A new *D. magna* specimen was used for each 12 minute experimental period.

Diphenhydramine

A generic, alcohol-free liquid allergy medication for children, distributed by Target Corporation (Minneapolis, MN), was the source of diphenhydramine hydrochloride. Other inactive ingredients listed on the medication label included citric acid, red food coloring, flavor, glycerin, high fructose corn syrup, poloxamer 307, purified water, sodium benzoate, sodium chloride, sodium citrate and sorbitol. *D. magna* were observed in 4.28 mM diphenhydramine. This was the undiluted concentration found in the medication.

Curcumin

Curcumin was obtained from commercial gelatin capsules marketed as Curcumin 95™ and manufactured by Jarrow Formulas (Los Angeles, CA). Each capsule contained approximately 76% curcumin, 18% curcumin II, 3% curcumin III and 3% cellulose. The inner contents of the capsules were added to water and filtered by gravity. The curcumin was filtered to remove large particles. A precise concentration of curcumin was determined by allowing the filtrate to evaporate and measuring the mass of curcumin remaining. *D. magna*

were observed in either a 3.62 µM or 7.24 µM concentration of curcumin. The filtrate was protected from light to avoid degrading the curcumin.

Measuring Heart Rate

The heart rates of *D. magna* were observed using a Nikon C-DS microscope. Individual *D. magna* were placed on concave microscope slides coated with petroleum jelly (Best Yet; Oklahoma City, OK) and kept in 200 µL of distilled water. Heart rate was measured twice in 2 minute intervals between 2-4 and 10-12 minutes after administering experimental compounds. *D. magna* in the control condition were kept in distilled water while experimental condition *D. magna* were administered appropriate concentration of curcumin, DPHM or both.

Statistical Analysis

The standard error of the mean was computed to determine the deviation of all data. Paired and unpaired two-tailed t-tests were used to determine the significance of the difference in heart rate between treatments and times respectively. A repeated measures ANOVA was used to determine significant interactions between factors. SPSS 15.0 and Microsoft Excel 2007 were used to conduct statistical analyses.

Illustrations

Adobe Illustrator CS3 was used to create figures for proposed mechanisms.

Results

The cardioactive effects of DPHM and curcumin were tested in *D. magna*. The mean heart rates of *D. magna* in the different treatment conditions between 2-4 minutes and 10-12 minutes are shown in Figure 1 and all values are reported in Table 1. The heart rate of untreated, control, *D. magna* (n = 30) did not change significantly between the 2-4 and 10-12 minute intervals ($p < 0.052$) based on student t-tests. All subject values (n) refer to individual *D. magna* used once for each test indicated. The heart rate of *D. magna* administered 3.62 µM curcumin (n = 6) did not change relative to the controls between 2-4 minutes ($p < 0.736$) or 10-12 minutes ($p < 0.557$). However, *D. magna* administered 7.24 µM curcumin (n = 20) showed a significant increase in heart rate relative to the controls between 2-4 minutes ($p <$ 6.78 E-6), and remained significantly higher between 10-12 minutes ($p < 2.13$ E-5).

The heart rate of *D. magna* administered 4.28 mM DPHM (n = 31) was not significantly different from the controls between 2-4 minutes ($p < 0.466$). However, the heart rate of DPHM treated *D. magna* was significantly lower than the controls during the 10-12 minute interval ($p < 0.0018$).

Table 1: Mean heart rate (bpm) of *D. magna*

	2-4 Min.	10-12 Min.
Control	215.6 ± 4.8	201.5 ± 5.4
Curcumin (3.67 µM)	211.1 ± 21.4	192.75 ± 19.5
Curcumin (7.24 µM)	254.4 ± 5.9	247.7 ± 8.9
DPHM	207.1 ± 10.3	192.7 ± 19.5
DPHM + Curcumin (3.67 µM)	184.0 ± 10.6	122.9 ± 10.9
DPHM + Curcumin (7.24 µM)	181.2 ± 11.8	111.9 ± 10.3

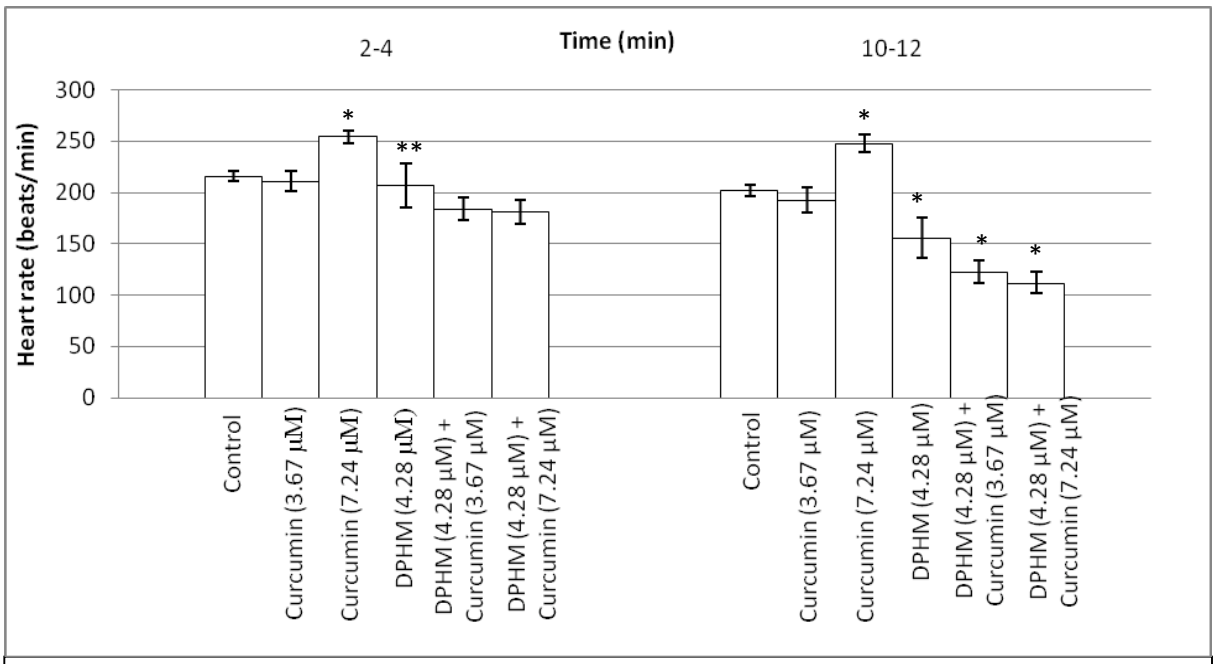

Figure 1. Mean heart rate (bpm) with standard error in for *D.magna* administered distilled water (controls), 3.62 µM and 7.24 µM dilutions of curcumin, 4.28 mM diphenhydramine (DPHM) and co-administered 4.28 mM DPHM and both curcumin concentrations. Heart rate taken between 2-4 minutes and 10-12 minutes after administration is shown. (*) indicates $p < 0.05$ compared to control within the same time interval. (**) indicates $p < 0.05$ between time intervals.

Co-administration of *D. magna* with 3.67 µM curcumin and 4.28 mM diphenhydramine (n = 33) slightly decreased the heart rate of *D. magna* at 2-4 minutes relative to the controls ($p < 0.01$) and greatly decreased the heart rate between 10-12 minutes relative to controls ($p <$ 4.57 E-8). Co-administration of *D. magna* with the 7.24 µM curcumin and 4.28 mM DPHM (n = 20) did not result in a significant change in heart rate between 2-4 minutes relative to co-administration with 3.67 µM curcumin ($p <$ 0.0036). Nor was a significant decrease seen in the 10-12 minute internal ($p <$ 6.58 E-11). Additionally, student t-tests demonstrated that there was no significant difference in heart rate between 2-4 minutes and 10-12 minutes when *D. magna* were administered 3.67 µM curcumin ($p < 0.541$) and 7.24 µM curcumin ($p < 0.535$).

An ANOVA revealed that DPHM had a significant effect on heart rate ($p < 0.001$). A significant interaction was also seen between time and DPHM ($p < 0.001$). No interaction between time and curcumin was found ($p <$ 0.543). Also, no significant effect was seen with curcumin alone ($p < 0.816$) or with curcumin and DPHM ($p < 0.374$). When 3.67 µM curcumin was not included in the ANOVA, a reduction in p value for curcumin alone ($p <$ 0.518), and curcumin and DPHM ($p < 0.167$) was noted. The interaction between curcumin, DPHM, and time was not significant with the 3.67 µM curcumin ($p < 0.995$) and without the 3.67 µM curcumin ($p < 0.951$), though a reduction in the p-value was seen.

Discussion

DPHM had a time-dependent negative chronotropic effect on *D. magna*, while curcumin had a positive chronotropic effect that was unchanged with time. The positive chronotropic effect of curcumin was not seen when co-administered with DPHM. ANOVA analysis did not find a significant interaction between curcumin and DPHM. This was likely due to the compounding of experimental error. Removing the lower curcumin concentration condition from the ANOVA improved the size of the effect of curcumin, though not within significance. However, a student's t-test suggested that significant differences existed between co-administration and single drug trials. These results significantly differed from the

hypothesized effects and indicate that the myogenic heart of *D. magna* is substantially different from the vertebrate heart.

DPHM was expected to have a positive chronotropic effect, given that the drug is known to induce tachycardia in humans and other mammalian animal models (Yap and Camm, 2002; de Abajo and Rodriguez, 2000). It is not clear why the reverse effect was seen in *D. magna*. It is possible that the anticholinergic effects of DPHM were somehow reduced in *D. magna* or simply not present with the concentration used. This is particularly worth noting given the ample evidence that acetylcholine and acetylcholinergic drugs produce similar results in *D. magna* as in other organisms with myogenic hearts (Baylor, 1942; Bekker and Krijgsman, 1951). It is also possible that the negative effects might not have been due to DPHM at all. The generic anti-allergy formula used in this study contained several inactive ingredients that could have confounded these results.

The positive chronotropic effect of curcumin in *D. magna* confirmed expectations. This effect appeared to occur within the 0-4 minute period after curcumin administration and was unchanged by 10-12 minutes. This indicated a fast acting, positive chronotropic role for curcumin that has not been previously reported. However, co-administration of DPHM unexpectedly negated this effect between 2-4 and 10-12 minutes.

While curcumin increased heart rate as expected, co-administeration with DPHM was not expected to negate this effect. If the positive chronotropic effects of curcumin were mediated through increased norepinephrine levels, as hypothesized, co-administration of DPHM would not have shown the effect found here. While curcumin had a positive chronotropic effect within two minutes of administration, DPHM did not have an effect until 10 to 12 minutes. Thus, if the positive chronotropic effect of curcumin was due to increased synaptic norepinephrine levels, co-administration with DPHM would not be expected to influence this effect between 2-4 minutes. This suggests that the positive chronotropic effect of curcumin and the negative chronotropic effects of DPHM were mediated by a common mechanism.

As noted previously, several studies have suggested that, unlike in vertebrates, norepinephrine is not the primary cardiac sympathetic neurotransmitter in *D. magna* (Villegas-Navarro et al., 2003; Postmes et al., 1989; Dzialowski et al., 2005). However, no studies have shown evidence for an alternative mode of sympathetic neurotransmission in *D. magna*. Based on the results of the current study, we propose that histamine may act as a sympathetic neurotransmitter in *D. magna* by binding to an H1-histamine receptor type homologue.

DPHM could have reduced heart rate by acting as an inverse agonist at the H1-histamine receptor. DPHM has only been shown to have a negative chronotropic effect in one other study in isolated rat atria (Pousti et al., 2003). However, these results have not been supported by other studies of DPHM in rats and other mammalian models (Liu et al., 2006; Simons, 2004). Differences between the interaction of DPHM and the muscarinic M2 acetylcholine receptor may explain the observed lack of a positive chronotropic effect in *D. magna*.

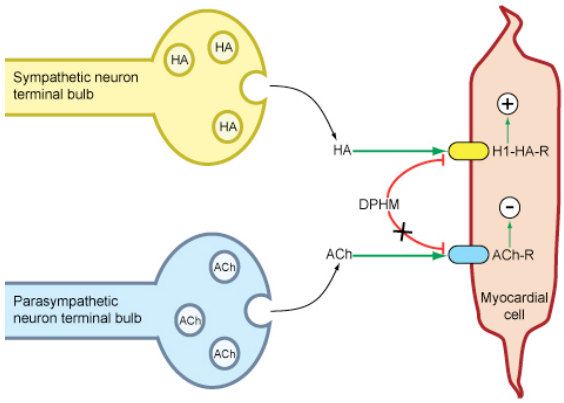

Figure 2. A proposed explanation for the cardioactive role of diphenhydramine (DPHM) in *Daphnia magna*. Histamine (HA) may be released by sympathetic cardiac neurons, producing a positive chronotropic effect by binding the H1-histamine receptor (H1-HA-R). DPHM is known to act as an inverse agonist at H1-HA-R. Thus, DPHM may prevent the sympathetic actions of HA. Unlike in mammalian models, DPHM may not antagonize the muscarinic acetylcholine receptor M2 (ACh-R) in *D. magna*. Thus, parasympathetic acetylcholine (ACh) may freely bind the ACh-R on myocardial cells and reduce heart rate.

Additionally, the inverse agonist effect of DPHM on the H1-histamine receptor may have produced the observed negative chronotropic effect. A proposed mechanism for the effects of DPHM on the heart of *D. magna* is shown in Figure 2.

Serotonin receptors on the heart have been found to modulate heart rate and other cardiac functions in rats (Bagdy et al., 1989). However, the negative chronotropic effects of DPHM probably did not result from the drug's activity as an SSRI. Curcumin has been found to increase serotonin levels as an MAOI potently (Xu et al., 2005; Kulkarni et al., 2008). Therefore, if serotonin had a negative chronotropic effect in *D. magna*, administration of curcumin would more likely have decreased heart rate, which was not the case.

It is possible that curcumin could prevent the degradation of histamine, resulting in increased levels of sympathetic histamine release. Numerous similar MAOIs inhibit histamine N-methyltransferase (HNMT), an enzyme involved in the metabolism of histamine (Boudikovi-Girard et al., 1993). However, the effects of curcumin on HNMT have not been investigated. A possible mechanism for the cardioactive effects of curcumin in *D. magna* is shown in Figure 3.

Several species of crustaceans express high levels of histamine on the heart and pericardial tissue (Fingerman et al., 1981). However, the role of the neurotransmitter in the heart of *D. magna* and other crustaceans is not well understood. Additionally, no study has examined the presence of histamine receptors in *D. magna* and only few G-protein coupled putative histamine receptors have been identified in invertebrates (Hamdan et al., 2002). Administration of histamine elevated the heart rate of the scorpion *Palamnaeus bengalensis*, which has a similar myogenic physiology to *D. magna* (Kanungo, 1957). However, histamine did not affect the myogenic heart of the crustacean species *Triops longicaudatus* (Yamagishi, 2003). Thus, previous studies do not indicate any clear role for histamine in the cardiac system of *D. magna*.

Relatively little research has been done on the interaction of curcumin and histamine.

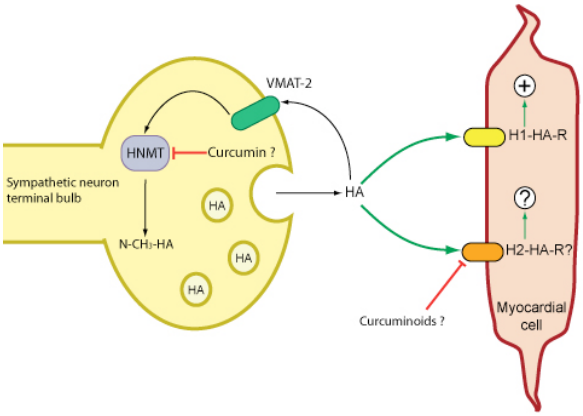

Figure 3. A proposed mechanism for the positive chronotropic effect of curcumin in *Daphnia magna*. Histamine (HA) produces a positive chronotropic effect (refer to Figure 2). HA is taken back up by the vesicular monoamine transporter-2 (VMAT-2) and either repackaged into synaptic vesicles or methylated by histamine N-methyl transferase (HNMT). Curcumin may antagonize HNMT, preventing HA methylation. Additionally, curcuminoids may antagonize the myocardial H2-histamine receptor (H2-HA-R). However, it is not clear how binding of endogenous HA to this receptor may affect the heart rate of *D.magna*.

However, a few studies have reported that curcumin antagonizes histamine responses in a variety of ways. Ram et al., (2003) found that curcumin attenuated allergic respiratory responses in guinea pigs. Curcumin has also been shown to inhibit the release of histamine *in vitro* through its antioxidative mechanisms (Suzuki et al., 2005). Extract of *Curcuma longa,* the plant from which turmeric and curcumin are derived, protected rats from gastric ulcers by antagonizing the H2-histamine receptor. While curcumin itself did not bind the receptor, it is possible that another curcuminoid in the extract did (Kim et al., 2005).

As this proposed mechanism for the action of curcumin is tentative, it should be noted that curcumin could potentially increase the heart rate of *D. magna* through other pathways. In addition, this mechanism may appear to contradict studies of the anti-inflammatory functions of curcumin (Ram et al., 2003). However, Suzuki et al. (2005) showed that the anti-histaminergic effects of curcumin were largely due to the compound's anti-oxidative function. Thus, curcumin could potentially act as an anti-inflammatory agent,

while also preventing the degradation of histamine in *D. magna* under non-oxidative stress conditions. Additionally, the inclusion of curcuminoids in the curcumin filtrate may have confounded the results of this study. These curcuminoids could have antagonized H2-histamine receptors in *D. magna*, as suggested in Kim et al. (2005). It is possible that curcuminoids affected the transduction of histamine responses in myocardial cells in a non-curcumin dependent way.

It has been speculated that histamine has a major role as a neurotransmitter in the sympathetic heart ganglia of vertebrates (Fryer, 2006). There is evidence that histamine may be released by sympathetic cardiac neurons and attenuate the release of norepinephrine and histamine by binding the H3-histamine receptor on cardiac sympathetic neuron terminals (Li et al., 2006; Imamura et al., 1994). Other studies have suggested that histamine and norepinephrine may both modulate cardiac sympathetic responses through joint negative feedback mechanisms (Krzan, 1996).

Histamine has been found to produce a positive chronotropic effect when directly administered to cardiac tissue in multiple animal models (Eckel et al., 1982). However, the effects of histamine appear to be modulated by different receptors in different animals. H2-histamine receptor agonists increased the heart rate of guinea pigs, though H1 receptor agonists had no effect (Flynn et al., 1979). However, Salata et al. (1995) found that the H1 receptor antagonist astemizole produced a negative chronotropic effect in anesthetized dogs. Additionally, Genovesse et al. (1988) found that the H1-histamine receptor mediated negative chronotropic and inotropic effects in human atrial myocardial cells. Thus, the functions of the H1 and H2-histamine receptors appear to differ between animal models.

The expression patterns of receptors in cardiac tissue also differ between animal models. (Flynn et al., 1979; Genovesse et al., 1988; Salta et al., 1995). Several mammals have been found to express very different distributions of H1 and H2 receptors on cardiac tissue (Matsuda et al., 2004). Little is known about the cardiac histamine receptor profile of myogenic crustaceans like *D. magna*. However, differences between mammals imply limitations in applying data across models. Further study of the localization of cardiac histamine receptors in crustaceans is necessary to resolve this issue.

The results of this study demonstrated that the H1-antihistamine DPHM exhibited an unexpected negative chronotropic effect on the heart rate of *D. magna.* Additionally, curcumin, a possible HNMT antagonist, produced a positive chronotropic effect. This effect was negated by DPHM. Based on these results, we propose that histamine may act as the primary cardiac sympathetic neurotransmitter in *D. magna*, possibly in lieu of norpeinephrine. However, the mechanisms described here are highly speculative. Future studies should seek to determine whether histamine changes the heart rate of *D. magna*, whether curcumin inhibits HNMT and how the interaction of DPHM and the muscarinic M2 acetylcholine receptor differs in *D. magna.* Better understanding of metabotropic histamine receptors in *D. magna* and crustaceans is also necessary. These studies should also test the effects of pure curcumin and DPHM to limit further sources of experimental error.

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References

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB (2007) Bioavailability of

Curcumin: Problems and Promises. *Molecular Pharmaceutics* 4: 807-818.

Bagdy G, Szemeredi K, Kanyicska B, Murphy DL (1989) Different Serotonin Receptors Mediate Blood Pressure, Heart Rate, Plasma Catecholamine and Prolactin Responses to m-Chlorophenylpiperazine in Conscious Rats. *J Pharmacol Exp Ther* 250**:** 72–78.

Baylor ER (1942) Cardiac Pharmacology of the Cladoceran Daphnia. Biological Bulletin 83: 165-172.

Bekker JM and Krijgsman BJ (1951) Physiological investigations into the heart function of Daphnia magna. *J Physiol* 115**:** 249–257.

Blumenthal MB, Wang HI, Markee S, Wang SC (1968) Effects of acetylcholine on the heart.*Am J Physiol* 214: 1280-1287.

Boudikovi-Girard B, Scott MC, Weinshilboum R (1993) Histamine N-methyltransferase: Inhibition by monoamine oxidase inhibitors. *Agents Actions* 40: 1-10.

Boyer EW, Shannon M (1995) The Serotonin Syndrome. *N Engl J Med* 352: 1112-1120.

Campbell AK, Wann KT, Matthews SB. (2004). Lactose causes heart arrhythmia in the water flea Daphnia pulex. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. *139*, 225-234.

Clark RF, Vance MV (1992) Massive diphenhydramine poisoning resulting in a wide-complex tachycardia: successful treatment with sodium bicarbonate. *Ann Emerg Med* 21: 318-21.

Cole GM, Teter B, Frautschy SA (2007) Neuroprotective effects of curcumin. *Advances in Experimental Medicine and Biology* 595: 197-212.

Crout JR, Creveling CR, Undenfriend S (1961) Norepinephrine metabolism in rat brain and heart. *J Pharmacol Exp Ther* 132: 269-277.

Dawson JK, Earnshaw SM, Graham CS (1995) Dangerous monoamine oxidase inhibitor interactions are still occurring in the 1990s. *Journal of Accident and Emergency Medicine* 12: 49-51.

de Abajo FJ, Rodriguez LA (2000) Risk of ventricular arrhythmias associated with nonsedating antihistamine drugs. *Br J Clin Pharmacol* 49:379-80.

Dzialowski EM, Turner PK, Brooks BW (2005) Physiological and Reproductive Effects of Beta Adrenergic Receptor Antagonists in Daphnia magna. *Arch Environ Contam Toxicol* 50: 503–510.

Eckel L, Gristwood RW, Nawrath H, Owen DAA, Satter P (1982) Inotropic and electrophysiological effects of histamine on human ventricular heart muscle. *J Physiol* 330:111-23.

Emadian SM, Caravati EM, Herr RD (1996) Rhabdomyolysis: a rare adverse effect of diphenhydramine overdose. *Am J Emerg Med* 14: 574-6.

Fahy P, Arnold P, Curry SC, Bond R (1989) Serial serum drug concentrations and prolonged anticholinergic toxicity after benztropine (Cogentin) overdose. *Am J Emerg Med* 7: 199-202.

Fingerman M, Nagabhushanam R, Sarojini R, Reddy PS (1994) Biogenic amines in crustaceans: identification, localization and roles. *Journal of Crustacean Biology* 14: 413-437.

Flynn SB, Gristwood RW, Owen DA (1979) Differentiation of the roles of histamine H1- and H2-receptors in the mediation of the effects of histamine in the isolated working heart of the guinea-pig. *Br J Pharmacol* 65: 127-137.

Fryer RM, Reinhart GA, Esbenshade TA (2006) Histamine in Cardiac Sympathetic Ganglia: A Novel Neurotransmitter? *Molecular Interventions* 65: 127-137.

Genovese A, Gross SS, Sakuma I, Levi R (1988) Adenosine Promotes Histamine-H1 mediated Negative Chronotropic and Inotropic Effects in Human Atrial Myocardium. *J Pharmacol Exp Ther* 247: 844-849.

Giacomini VA, Reis CC (1986) Antagonistic effect between histamine and norepinephrine on isolated guinea-pig right atrium. *Gen Pharmacol* 17: 219-225.

Goel A, Kunnumakkara AB, Aggarwal BB (2008) Curcumin as "Curecumin": From kitchen to clinic. *Biochemical Pharmacology* 75: 789-809.

Imamura M, Poli E, Omoniyi AT, Levi R (1994) Unmasking of of Activated Histamine-H3 Receptors in Myocardial Ischemia: Their Role as Regulators of Exocytotic Norepinephrine Release. *J Pharmacol Exp Ther* 271: 1259-1266.

Ishikawa T, Hume JR, Keef KD (1993) Modulation of K+ and Ca2+ channels by histamine H1-receptorstimulation in rabbit coronary artery cells. *Journal of Physiology* 468:379-400.

Jiao Y, Wilkinson J, Pietch EC, Buss JL, Wang W, Planalp R, Torti FM, Torti SV (2006) Iron chelation in the biological activity of curcumin. *Free Radical Biology and Medicine* 40: 1152-1160.

Jones J, Dougherty J, Cannon L (1986) Diphenhydramine-induced toxic psychosis. *Am J Emerg Med* 4:369-71.

Kanungo MS (1957) Cardiac Physiology of the Scorpion Palamneaus Bengalensis C. Koch. *The Biological Bulletin* 113: 135-140.

Kim DC, Kim SE, Choi BH, Baek NI, Kim D, Kim MJ, Kim KT (2005) Curcuma longa Extract Protects against Gastric Ulcers by Blocking H2 Histamine Receptors. *Biological and Pharmaceutical Bulletin* 28: 2200-2224.

Kirk MA (1998) Anticholinergics and antihistamines. In: Haddad LM, Shannon MW, Winchester JF, eds. Clinical Management of Poisoning and Drug Overdose. 3rd ed. WB Saunders Co; 1998:641-9.

Koppel C, Ibe K, Tenczer J (1987) Clinical symptomatology of diphenhydramine overdose: an evaluation of 136 cases in 1982 to 1985. *J Toxicol Clin Toxicol* 25:53-70.

Krzan M (1996) Histamine receptors in the heart- Molecular characteristics, physiology and pharmacology. *Inflammopharmacology* 4: 241-257.

Kunnumakkara AB, Anand P, Aggarwal BB (2008) Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Letters* 269: 199-225.

Kulkarni SK, Bhutani MK, Bishnoi M (2008) Antidepressant activity of curcumin: involvement of serotonin and dopamine system. *Psychopharmacology* 201: 435-442.

Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, Boggs ME, Crowell J, Rock CL, Brenner DE (2006) Dose escalation of a curcuminoid formulation. *BMC Complementary and Alternative Medicine* 6:10-4.

Leurs R, Church MK, Taglialatela M (2002) H1-antihistamines: inverse agonism, anti-inflammatory actions and cardiac effects. *Clin Exp Allergy* 32: 489–98.

Li M, Hu J, Chen Z, Meng J, Wang H, Ma X, Luo X (2006) Evidence for histamine as a neurotransmitter in the cardiac sympathetic nervous system. *Am J Physiol Heart Circ Physiol* 291: H45-H51.

Litovitz TL, Klein-Schwartz W, White S, Cobaugh DJ, Youniss J, Drab A, Benson BE (2000) 1999 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am J Emerg Med* 18: 517-574.

Liu H, Zheng Q, Farley JM (2006) Antimuscarinic actions of antihistamines on the heart. *Journal of Biomedical Science* 13: 395–401.

Matsuda N, Jeamin S, Takahashi Y, Hatta E, Kobayashi M, Matsuyama K, Kawakami N, Sakuma I, Gando S, Fukui H, Hattori Y, Levi R (2004) Histamine H-1 and H-2 receptor gene and protein levels are differentially expressed in the hearts of rodents and humans. *J Pharmacol and Exp Ther* 209: 786-95.

Mitsuchashi M, Payan DG (1989) Molecular and cellular analysis of histamine H1 receptors on cultured smooth muscle cells. *J Cell Biochem* 40: 183–92.

Naito M, Wu X, Nomura H, Kodama M, Kato Y, Kato Y, Osawa T (2002) The Protective Effects of Tetrahydrocurcumin of Oxidative Stress in Cholesterol-fed Rabbits. *Journal of Artherosclerosis and Thrombosis* 9: 243-250.

Postmes TJ, Prick R, Brorens I (1989) The deceleration of the heart frequency in the waterflea Daphnia magna by adrenoceptor agonists and antagonists. *Hydrobiolgia* 171: 141-148.

Pousti A, Malihi G, Bakhtiarian A, Abdollahi Z (2003) The comparative effects of four

antihistamines isolated rat atria. *Iranian J Pharmacol and Ther* 1: 20-3.

Peret-Almeida LP, Cherubino APF, Alves RJ, Duffosé L, Glória MBA (2005) Seperation and determination of the physico-chemical characteristics of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Research International* 38: 1039-1044.

Ram A, Das M, Ghosh B (2003) Curcumin Attenuates Allergen-Induced Airway Hyperresponsiveness in Sensitized Guinea Pigs. *Biol Pharm Bull* 26: 1021-1024.

Reiner PB, Kamondi A (1994) Mechanisms of antihistamine induced sedation in the human brain: H-1 receptor activation reduces a background leakage potassium current. *Neurosci* 59:579-588.

Richmond M, Seger D (1985) Central anticholinergic syndrome in a child: a case report. *J Emerg Med* 3:453-6.

Rodriguez MJ, Saura J, Billett EE, Finch CC, Mahy N (2001) Cellular localization of monoamine oxidase A and B in human tissue outside of the central nervous system. *Cell Tissue Res* 304: 215–220.

Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R (1995) Anti-tumor and antioxidant activity of natural curcuminoids. *Cancer Letters* 94: 79-83.

Ryu EK, Choe YS, Lee KH, Choi Y, Kim BT (2006) Curcumin and Dehydrozingerone Derivatives: Synthesis, Radiolabeling and Evaluation for ß-Amyloid Plaque Imaging. *J Med Chem* 49: 6111-6119.

Salata JJ, Jurkiewicz NK, Wallace AA, Stupienski RF, Guinosso PJ, Lynch JJ (1995) Cardiac electrophysiological actions of the histamine H-1 receptor antagonists astemizole and terfenadine compared with chlorpheniramine and pyrilamine. *Circulation Research* 76:110-9.

Serio RN. (2004). Acute delirium associated with combined diphenhydramine and linezolid use. *Ann Pharmacother* 38: 62-5.

Simons FE (2004) Advances in H1-antihistamines. *N Engl J Med* 351: 2203–17.

Spicer JI (2001) Development of cardiac function in crustaceans: patterns and proceses. *Amer Zool* 41: 1068-1077.

Strempel S, Schroeder C, Hemmersbach R, Boese A, Tank J, Diedrich A, Heer M, Luft FC, Jordan J (2008) Norepinephrine transporter inhibition alters the hemodynamic response to hypergravitation. *J Appl Physiol* 104: 756–760.

Stearns SC (1975) Light Responses of Daphnia pulex. *Limnology and Oceonagraphy* 20: 564-570.

Stein RJ, Richter WR, Zussman RA, Brynjolfsson G (1966) Ultrastructural Characterization of *Daphnia* Heart Muscle. *J Cell Biol* 29: 168-170.

Suzuki M, Nakamura T, Iyoki S, Fujiwara A, Watanabe Y, Mohri K, Isobe K, Ono K, Yano S (2005) Elucidation of anti-allergic activities of curcumin-related compounds with a special reference to their anti-oxidative activities. *Biol Pharm Bull* 28: 1438-1443.

Sype JW, Khan IA (2005) Prolonged QT interval with markedly abnormal ventricular repolarization in diphenhydramine overdose. *Int J Cardiol* 99: 333-5.

Taglialatela M, Timmerman H, Annunziato L (2000) Cardiotoxic potential and CNS effects of first-generation antihistamines. *Trends in Pharmacol Sci* 21:52-6.

Thakur AC, Aslam AK, Aslam AF, Vasavada B, Sacchi T, Kahn I (2005) QT interval prolongation in diphenhydramine toxicity. *Int J Cardiol* 98:341-3.

Villegas-Navarro AV, Rosas-L E, Reyes JL (2003) The heart of Daphnia magna: effects of four cardioreactive drugs. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 136: 127-134.

Weber WM, Hunsaker LA, Abcouwer SF, Deck LM, Vander Jagt DL (2005) Anti-oxidant effects of curcumin and related enones. *Bioorganic and Medicinal Chemistry* 13: 3811-3820.

Weisman RS (1998) Antihistamines. In: Goldfrank LR, ed. Goldfrank's Toxicologic Emergencies. 6th ed. McGraw-Hill; 1998:603-9.

Wolff AA, Levi R (1986) Histamine and cardiac arrythmias. *Circulation Research* 58: 1-16.

Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, Li ZJ (2005) The effects of curcumin on

depressive like behaviors in mice. *European Journal of Pharmacology* 518: 40-46.

Yamagishi H (2003) Aminergic Modulation of the Myogenic Heartin the Branchiopod Crustacean Triops longicaudatus. *Zoological Science* 20: 841-846.

Yap YG, Camm AJ (2002) Potential cardiac toxicity of H1-antihistamines. *Clin Allergy Immunol* 17: 389-419.