Left Ventricular Myocardial Contractile Response to Aortic Constriction in the Hyperthyroid Guinea Pig

By M. Jay Goodkind, M.D.

ABSTRACT

Maximum left ventricular systolic pressure response to aortic constriction served as a measure of myocardial contractility in anesthetized euthyroid and hyperthyroid guinea pigs. This response in hyperthyroid guinea pigs (155.4 ± 1.40 mm Hg) was significantly greater than in euthyroid animals (147.3 ± 1.33 mm Hg) (P < .001) and developed at a lower end-diastolic pressure in hyperthyroid (11.27 ± 0.418 mm Hg) than in euthyroid guinea pigs (14.15 ± 0.521 mm Hg) (P < .001). Beta-adrenergic receptor blockade with propranolol decreased the heart rate in both groups of animals but did not alter the contractile response. After propranolol, the heart rate of the hyperthyroid animals, though decreased, was still greater than that of euthyroid controls. Cardiac stimulation at a rate similar to that of the hyperthyroid animals (430 beats/min) did not change the left ventricular contractile response of normal guinea pigs. It is concluded that myocardial contractility in hyperthyroid guinea pigs is greater than normal. Increased activity of the sympathetic nervous system is probably present in hyperthyroidism, is reflected in the tachycardia, but does not appear to increase myocardial contractility. The increased myocardial contractility therefore is probably due in large part to a direct effect of thyroid hormone on cardiac muscle.

ADDITIONAL KEY WORDS propranolol hemodynamic function beta-receptor blockade pressure work

tachycardia cardiac stimulation

Many attempts have been made to define the relationship of the sympathetic nervous system to the cardiovascular changes produced by excess thyroid hormone. For years the hyperkinetic circulation of thyrotoxicosis was thought to be related to a hypersensitivity to catecholamines (1). However, recent reports have shown that thyroxine does not increase the pressor, chronotropic, or inotropic effects of administered catecholamines in humans (2), dogs (3, 4), cats (5), or rats (4, 6). Augmentation of sympathetic nervous system activity in hyperthyroidism has also been proposed as a mechanism for the cardiovascular effects of thyroid hormone (1). If thyroid hormone causes an increase in sympathetic nervous system activity, both an increased heart rate and an increased myocardial contractility should be present in thyrotoxic animals. However, increased myocardial "contractility" has not been clearly demonstrated in thyroid-stimulated animals. In vitro, the isometric myocardial contractile force of thyrotoxic animals has been found by some to be less than (4, 7-9) and by others to be greater than that of normal controls (10). In vivo, the thyroid-stimulated heart responds to an increased venous return with greater than normal stroke volume and stroke...
work (11). This latter finding is compatible with an increase in myocardial contractility.

The studies to be described were designed to measure in vivo the myocardial contractility of the left ventricle in hyperthyroid guinea pigs and to compare the results with those obtained in normal control animals. Myocardial contractility was characterized by the maximum left ventricular systolic pressure attained in response to graded aortic constriction.

Methods

Adult male guinea pigs of the Hartley strain (400-1000 g) were fed standard Purina guinea pig feed and water ad libitum with supplements of lettuce. Thyrotoxicosis was produced by the daily intraperitoneal injection of sodium L-thyroxine (100 μg/day for 7 to 10 days). On this regimen the animals lost weight and developed tachycardia and left ventricular hypertrophy (12). They were studied at the end of thyroxine treatment, and the results were compared to those of similar studies in normal controls.

Anesthesia was achieved with Dial-urethane (0.6 ml/kg) intraperitoneally. A tracheal cannula was inserted and connected to a Palmer small-animal respirator which delivered a gas mixture of 95% O₂—5% CO₂. Arterial pH, monitored during some of the experiments, changed only when left ventricular pressure and response to aortic constriction decreased. Results from such animals were discarded. The initial arterial blood pH of euthyroid guinea pigs was 7.40 ±0.011 and of hyperthyroid animals 7.38 ± 0.015. The difference between these two groups was not significant.

Through a midsternal thoracotommy, the pericardium was removed and a cotton suture placed about the ascending aorta. The free ends of the loop were threaded through the eye of an aneurysm needle whose convex curve rested on the aorta. Pulling on the free ends reduced the size of the loop, producing graded compression of the ascending aorta against the aneurysm needle without displacement of the aorta. Continued pull resulted in almost complete occlusion of the aorta.

After hemostasis was achieved, sodium heparin (500 units) was administered through a 19-gauge, thin wall needle placed through the apex into the chamber of the left ventricle. Smaller supplemental doses of heparin were given at intervals of 45 to 60 minutes during the experiments. This needle was also used as a cannula for measuring pressure with an attached Statham P-23Gb strain gauge. Simultaneous low and high sensitivity tracings for systolic and end-diastolic pressure respectively were recorded with carrier amplifiers and an oscillographic recorder. The system had a natural frequency response of 35 cps. The maximal rate of rise of systolic pressure (dP/dt max) was measured initially by direct measurement of tracings taken at a paper speed of 200 mm/sec and later with an electronic differentiator. The differentiator was an operational amplifier with a linear gain frequency response.

Throughout this paper the phrases “systolic pressure” and “end-diastolic pressure” refer to left ventricular pressures.
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Data were evaluated with Student's $t$-test for unpaired data. When animals served as their own controls, the $t$-test for paired data was used. The propranolol study to be described was evaluated both by the $t$-test for paired and unpaired data, and by a 2 x 2 factorial design for experiments with unequal cell size (12). Results were considered significant if the $P$ value was less than or equal to 0.05.

Results

LEFT VENTRICULAR RESPONSE TO GRADED AORTIC CONSTRICTION

The three phases of left ventricular compensation in response to graded aortic constriction are: (1) compensation when systolic pressure rises with either a transient increase or no change in end-diastolic pressure; (2) partial compensation in which end-diastolic pressure rises slightly in association with a continued rise of systolic pressure; and (3) decompensation characterized by a continued rise of end-diastolic pressure as systolic pressure remains constant or decreases. In practice, phase 1 is passed through rapidly and phase 3 is not permitted to develop to its full extent in order to avoid prolonging the stress on the left ventricular myocardium.

A typical response of systolic and end-diastolic pressures to graded aortic constriction is shown in Figure 1. At the beginning of phase 1, the aorta was gradually constricted by tightening the loop around it. The constriction was released as soon as phase 3 began. Systolic pressure usually rose concomitantly with onset of aortic constriction. The systolic pressure rise of phase 1 in Figure 1 is not impressive because the initial aortic constriction was not sufficient. During graded aortic constriction the myocardium of normal guinea pigs could not compensate adequately.

Changes in Left Ventricular Function in Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid (N = 6)</th>
<th>Hypothyroid (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure</td>
<td>$-5.7 \pm 3.04$</td>
<td>$+3.9 \pm 3.17$</td>
</tr>
<tr>
<td>End-diastolic pressure</td>
<td>$-0.6 \pm 0.26$</td>
<td>$-0.8 \pm 0.27$</td>
</tr>
<tr>
<td>$dP/dt_{max}$</td>
<td>$-301 \pm 306.1$</td>
<td>$-138 \pm 272.3$</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>$+0.5 \pm 5.39$</td>
<td>$+21.9 \pm 8.39$</td>
</tr>
<tr>
<td>Maximum systolic pressure during aortic constriction (mm Hg)</td>
<td>$-8.7 \pm 3.80$</td>
<td>$-6.3 \pm 2.62$</td>
</tr>
<tr>
<td>End-diastolic pressure during aortic constriction (mm Hg)</td>
<td>$-2.4 \pm 1.62$</td>
<td>$-2.6 \pm 1.19$</td>
</tr>
</tbody>
</table>

All values are expressed as mean difference ± SE of the difference between control measurements and values 24 hours later.

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constriction, about 10 to 15 seconds were required to achieve a maximum left ventricular systolic pressure response. Cardiac arrhythmias were more likely to occur if aortic constriction was produced too rapidly; data were not used if the maximum systolic pressure was preceded by arrhythmia. Two to five minutes were allowed for recovery between constrictions; recovery was assumed when systolic and end-diastolic pressures returned to the preconstriction level. A graph of the stepwise rise in systolic pressure on the ordinate against the corresponding end-diastolic pressure on the abscissa during phase 2 results in a curve similar to that previously obtained in dogs (14). The four curves shown in Figure 2 demonstrate the reproducibility of this response over a 30-minute period in a single animal. Myocardial contractile ability was characterized by the maximum left ventricular systolic pressure achieved during aortic constriction. The mean values for maximum systolic pressure were used for a statistical comparison of the myocardial contractility of normal and hyperthyroid animals.

A study that included administration of a drug or cardiac stimulation required from 2½ to 3 hours from the time the animal's thorax was opened to completion. As a control, nine normal and eight hyperthyroid guinea pigs were followed for 2½ hours with measurements every half hour of the left ventricular response to aortic constriction. During the 2½ hours of observation, no significant change was found in any of the variables measured (Table 1).

FIGURE 3

Left ventricular hemodynamic functions in euthyroid and hyperthyroid guinea pigs. Each bar represents the mean, and the vertical lines represent ± 1 SEM. Numbers in parentheses above bars represent the P values obtained by statistical comparison of results from hyperthyroid with those from euthyroid animals by t-test for unpaired data. N.S. = not significant. See Figure 1 for abbreviations.
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609

120
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(8)
£1 60
• HYPCRTHYROID (28)
—- EUTHYROID (25)

FIGURE 4
Average pressure curves for euthyroid and hyperthyroid guinea pigs. Each point represents the mean of that group. The vertical lines represent ± 1 SEM. See Figure 1 for abbreviations.

The contractile response to aortic constriction was studied in normal and hyperthyroid guinea pigs (Figs. 3-5). All of the data used for comparison were the maximum values obtained within the first hour of study in each animal. Recorded dP/dt\text{max}, heart rate, and systolic pressure were higher than normal in hyperthyroid animals (Fig. 3). No difference in end-diastolic pressure was noted between the two groups. Body temperature of hyperthyroid animals (39.4±0.11°C) was greater than that of normal (38.0±0.09°C) (P<.001).

The average pressure curves during aortic constriction for 27 euthyroid and 28 hyperthyroid animals are depicted in Figure 4. At any given end-diastolic pressure, hyperthyroid animals developed a significantly higher systolic pressure than normal controls during phase 2 of the left ventricular response to aortic constriction. Maximum systolic pressures and their corresponding end-diastolic pressures were obtained from the responses of 112 euthyroid and 75 hyperthyroid animals in the two groups (Fig. 5). Left ventricles of hyperthyroid guinea pigs achieved a greater maximum systolic pressure than normal animals, and this occurred at a correspondingly lower average end-diastolic pressure.

EFFECT OF BETA-ADRENERGIC RECEPTOR BLOCKADE

Propranolol\textsuperscript{3} (50 µg/kg) was given slowly by direct infusion into the left ventricle to evaluate the role of the sympathetic nervous system in the myocardial response to aortic constriction in hyperthyroid guinea pigs. Administration of 75 µg/kg was associated with a marked myocardial depression and more than 50% mortality. Hemodynamic responses to 50 µg/kg were compared in 19 normal and 14 hyperthyroid animals (Table 2). The dosage selected caused a decrease in the positive chronotropic effect of isoproterenol (0.5 µg/}

\textsuperscript{3}Generously supplied by Ayerst Laboratories, New York, N. Y.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure4.png}
\caption{Average pressure curves for euthyroid and hyperthyroid guinea pigs.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure5.png}
\caption{Maximum left ventricular systolic pressure (LVSP) and end-diastolic pressure (LVEDP) response to aortic constriction in euthyroid and hyperthyroid guinea pigs.}
\end{figure}

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Left ventricular hemodynamic function in euthyroid guinea pigs before and during right ventricular stimulation at 430 beats/min. Asterisks indicate data significantly different from control levels. Abbreviations as in Figures 3 and 5.

TABLE 2
Effects of Propranolol on Left Ventricular Function of Euthyroid and Hyperthyroid Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>(1) Control</th>
<th>(2) Propranolol</th>
<th>(4) Difference between propranolol and control</th>
<th>(5) Elapsed-time controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>E: 65 ± 1.3</td>
<td>64 ± 1.5</td>
<td>-1.1 ± 1.63</td>
<td>-1.0 ± 3.10</td>
</tr>
<tr>
<td></td>
<td>H: 92 ± 2.5</td>
<td>90 ± 2.6</td>
<td>-1.7 ± 2.11</td>
<td>+0.3 ± 2.68</td>
</tr>
<tr>
<td>End-diastolic pressure</td>
<td>E: 4.3 ± 0.33</td>
<td>3.9 ± 0.35</td>
<td>-0.4 ± 0.23†</td>
<td>-0.3 ± 0.32</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>H: 5.0 ± 0.55</td>
<td>4.7 ± 0.59</td>
<td>-0.4 ± 0.25†</td>
<td>-0.4 ± 0.11†</td>
</tr>
<tr>
<td>dP/dt_max (mm Hg/sec)</td>
<td>E: 2044 ± 98.3</td>
<td>1785 ± 85.2</td>
<td>-260 ± 66.1†</td>
<td>+43 ± 172.3</td>
</tr>
<tr>
<td></td>
<td>H: 3960 ± 262.4</td>
<td>3736 ± 244.2</td>
<td>-231 ± 117.5†</td>
<td>-131 ± 94.3†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>E: 287 ± 5.3</td>
<td>275 ± 6.1</td>
<td>-13 ± 4.3†</td>
<td>+4 ± 3.8†</td>
</tr>
<tr>
<td></td>
<td>H: 413 ± 8.4</td>
<td>405 ± 7.5</td>
<td>-8 ± 3.7†</td>
<td>+9 ± 5.0‡</td>
</tr>
<tr>
<td>Maximum systolic pressure during aortic constriction (mm Hg)</td>
<td>E: 130 ± 3.3</td>
<td>130 ± 4.0</td>
<td>-9.2 ± 3.25†</td>
<td>-1.4 ± 3.29†</td>
</tr>
<tr>
<td></td>
<td>H: 151 ± 3.8</td>
<td>144 ± 2.8</td>
<td>-6.5 ± 1.34†</td>
<td>-1.4 ± 2.10</td>
</tr>
<tr>
<td>End-diastolic pressure during aortic constriction (mm Hg)</td>
<td>E: 14.8 ± 1.25</td>
<td>13.8 ± 1.17</td>
<td>-1.0 ± 1.01</td>
<td>-1.7 ± 1.58</td>
</tr>
<tr>
<td></td>
<td>H: 11.9 ± 1.04</td>
<td>11.0 ± 0.97</td>
<td>-0.9 ± 0.76</td>
<td>-1.0 ± 0.30†</td>
</tr>
</tbody>
</table>

Column 1: Propranolol-treated animals—euthyroid (E), N = 19; hyperthyroid (H), N = 14; Elapsed-time controls—euthyroid, N = 7; hyperthyroid, N = 8. Data in Columns 2 and 3 are expressed as means ± SEM and data in Columns 4 and 5 are expressed as mean differences ± SE of the difference.

*Significant difference by paired t-test (P ≤ .05).
†Significant difference between net propranolol effect and time effect by t-test for unpaired data (P ≤ .05).
‡Significant difference between propranolol effect and time effect by analysis of variance (P ≤ .05).
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kg) in 4 of 6 normal and all of 6 hyperthyroid guinea pigs without reducing the systolic pressure. The maximum response of systolic pressure to this dose of isoproterenol was reduced after propranolol by 25% in 4 of 5 normal animals. All doses of propranolol were given over a 5-minute period and were diluted in 0.9% NaCl; doses were such that each animal received 1 ml of saline/kg. Studies of left ventricular contractility were made 30 minutes after administration of the drug. Each animal's response to β-adrenergic receptor blockade was compared to its own control studies before drug administration, and the differences compared by the t-test for paired data (Table 2). In addition, the changes in function after propranolol were compared to the average changes observed in the controls described (see Methods) by analysis of variance and by t-test for unpaired data. The results from the control animals were taken during the 1½ to 2 hours after the thorax was opened; this corresponded to the time the propranolol-treated animals were studied.

Heart rate decreased more in the 30 minutes after propranolol than with lapse of time alone in both euthyroid and hyperthyroid animals. Other hemodynamic functions (systolic pressure, end-diastolic pressure, dP/dt max), though reduced from pre-drug values in these animals, did not change more than in the controls. The maximum systolic pressure response to aortic constriction decreased after propranolol from the pre-drug values in both the euthyroid and hyperthyroid group (P < .05). However, when the results of propranolol administration were compared with controls by analysis of variance, only the heart rate in euthyroid and hyperthyroid animals was found to be significantly reduced by the drug. The direct cause of the reduced heart rate cannot be definitely determined since the analysis of variance indicated a significant interaction.

EUTHYROID GUINEA PIG HEARTS STIMULATED AT 430 BEATS PER MINUTE

The average heart rate of the hyperthyroid animals was approximately 120 beats/min faster than that of the euthyroid controls. To evaluate the role of the tachycardia on the maximum systolic pressure response to aortic constriction in the hyperthyroid animals, 18 normal guinea pigs were studied before and during cardiac pacing at 430 beats/min. The results are shown in Figure 6. A slight but significant decrease in systolic pressure occurred at the rapid heart rate, without significant change in end-diastolic pressure. At the higher heart rate, dP/dt max decreased. Despite the reduction in the maximum rate of rise of pressure, the maximum pressure response and its end-diastolic pressure during aortic constriction was not affected by the rapid heart rate.

Discussion

The maximum increase in left ventricular systolic pressure during aortic constriction, used as an index of myocardial contractility, was greater in the hyperthyroid guinea pig than in normal control animals. This method for examining myocardial contractility provides a relatively stable and reproducible measure in vivo for as long as 3 hours. The maximum left ventricular pressure achieved in guinea pigs correlates well with the adenosine triphosphate content of the myocardium (15). In normally coupled oxidative phosphorylation, the adenosine triphosphate concentration parallels myocardial oxygen consumption and myocardial work. The pressure response of the left ventricle gives an index of cardiac performance which follows expected changes in myocardial function in dogs as represented by the conventional Starling curve of ventricular function (14). Maximum left ventricular systolic pressure achieved during aortic constriction, obtained by an almost pure isovolumic contraction, approximates the isometric "absolute strength" of the myocardium described by Frank (16). The pressure response to increased outflow resistance is therefore considered a valid index of myocardial contractility.

Baroreceptor reflex release of sympathetic nervous system stimuli does not appear to contribute significantly to the left ventricular response to aortic constriction. Previous
studies of aortic constriction in the dog indicated that aortic pressure distal to the obstruction falls only near the point of maximal obstruction. At this point, the loop must distort pressure receptors in the wall of the aorta and produce a drop in pressure in the aortic arch and in the carotid sinuses, but the effect of these changes was judged to be quite small (14). Absence of any consistent change in heart rate during aortic constriction and lack of significant change in maximum systolic pressure after propranolol in the guinea pig also suggests that a major reflex response of the sympathetic nervous system does not play an important role in the pressure responses that occurred following aortic constriction.

Some degree of hyperactivity of the sympathetic nervous system is probably present in the hyperthyroid guinea pigs of the present studies and in the hyperthyroid patients studied by Howitt and Rowlands (17), since β-adrenergic receptor blockade reduced the heart rates of these subjects. A greater than normal myocardial concentration of norepinephrine and uptake of circulating norepinephrine in the hyperthyroid guinea pig (12) is also compatible with an increase in sympathetic activity. Failure of propranolol to reduce heart rates of hyperthyroid subjects to normal levels suggests, however, that the tachycardia may also be due to a direct effect of thyroid hormone on the cardiac pacemaker, or to an effect secondary to the hormone's action at a site distant from the heart. Lack of effect of propranolol on the left ventricular contractile response to aortic constriction indicates either that the increased contractility observed in the hyperthyroid animals is not the result of increased sympathetic activity or that the myocardial adrenergic receptor for an isotropic stimulus differs in some way from the receptor for a chronotropic stimulus. A significantly increased contractility of papillary muscles from hyperthyroid cats and from hyperthyroid cats treated with reserpine to deplete myocardial norepinephrine stores also suggests that the change in contractility is not a result of increased sympathetic activity (10). It should be pointed out that the myocardial depressant effect of propranolol cannot be ruled out as a cause of the reduced heart rate in the present studies. However, lack of effect of the drug on the systolic pressure and on contractile response of the left ventricle is evidence against such a depression. The changes resulting from β-adrenergic receptor blockade suggest then that increased sympathetic nervous activity is present in hyperthyroidism, but has only a slight chronotropic and no significant isotropic effect on cardiac function.

The mechanism for an increased myocardial contractility in the hyperthyroid guinea pig remains to be defined. Four factors may play a role. First, the increased heart rate may serve as a positive inotropic agent in hyperthyroidism. However, the present study demonstrates that an acute increase in heart rate in normal guinea pigs to a level similar to that of the hyperthyroid state failed to produce a positive inotropic response as measured by changes in maximum systolic pressure. The maximum rate of rise of systolic pressure, a measure of myocardial contractile function, actually decreased in animals stimulated at 430 beats/min. Hemodynamic effects of an acute increase in heart rate may not be entirely comparable to those of the chronic tachycardia of thyrotoxicosis. Also, direct electrical pacing of the apical ventricular myocardium is not physiological and may raise other problems such as the route taken by the wave of myocardial depolarization. The disparity between the decrease in heart rate and the lack of change in maximum systolic pressure after propranolol suggests that the two are not related. In addition, the in vitro studies of Buccino et al. (10), which demonstrated an increased contractility in hyperthyroidism, were performed at fixed rates of stimulation, thus obviating heart rate as a major factor in the contractile response. Some effect of heart rate on myocardial contractility of the hyperthyroid subject cannot be ruled out, but its role does not appear to be of prime importance.

A slightly higher than normal body tempera-
ture in the hyperthyroid guinea pig may influence myocardial contractile ability. In vitro atrial myocardial contractility decreases as temperature is increased in tissue from both hyperthyroid and normal rats (8). A higher than normal body temperature of hyperthyroid guinea pigs would therefore tend to diminish rather than accentuate any difference in myocardial contractility between normal and hyperthyroid animals.

Myocardial hypertrophy associated with hyperthyroidism is a third factor which may alter contractility. Preliminary studies in this laboratory indicate that contractility in hypertrophied guinea pig hearts is normal. The work of others suggests that the contractility of hypertrophied myocardium may be increased, normal, or decreased (18). Maximum left ventricular work obtainable from rats with left ventricular hypertrophy secondary to chronic aortic constriction is less than that observed in hyperthyroid rats with a similar degree of myocardial hypertrophy (11). It is apparent that more studies must be made of the function of hypertrophied hearts, though hypertrophy probably does not contribute significantly to the myocardial contractility of the hyperthyroid animal.

Lastly, a direct effect of thyroxine on the heart may be a mechanism for an increased myocardial contractility in hyperthyroidism. The exact nature of this effect remains to be determined. However, it is known that thyroxine has a direct metabolic effect on the myocardium which leads, for example, to a stimulation of protein anabolism (19) and a change in the primary energy source used by heart muscle from one of carbohydrates to one of fatty acids (20). In addition, thyroxine may also have a direct effect on the cell membrane to alter transport of electrolytes and result in changes in myocardial contractility (21). Finally, the increased myocardial contractility of hyperthyroid animals is demonstrable in vitro, without nervous and humoral influences (10). Thus, a direct effect of thyroid hormone on cardiac muscle appears to be the most likely mechanism to explain the increased myocardial contractility observed in the hyperthyroid animal.

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**References**


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