Histamine H₃ activation depresses cardiac function in experimental sepsis

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Histamine H₃ activation depresses cardiac function in experimental sepsis. J. Appl. Physiol. 85(5): 1693–1701, 1998.—In the heart, histamine (H₃) receptors may function as inhibitory presynaptic receptors that decrease adrenergic norepinephrine release in conditions of enhanced sympathetic neural activity. We hypothesized that H₃-receptor blockade might improve cardiovascular function in sepsis. In a canine model of Escherichia coli sepsis, we found that H₃-receptor blockade increased cardiac output (3.6 to 5.3 l/min, P < 0.05), systemic blood pressure (mean 76 to 96 mmHg, P < 0.05), and left ventricular contractility compared with pretreatment values. Plasma histamine concentrations increased modestly in the H₃-blocker–sepsis group compared with values obtained in a nonsepsis–time-control group. In an in vitro preparation, histamine H₃ activation could be identified under conditions of septic plasma. We conclude that activation of H₃ receptors may contribute to cardiovascular collapse in sepsis.

cardiac depression; septic shock; sympathetic response

In the cardiovascular system, evidence has accumulated that histamine (H₃) receptors may function as inhibitory presynaptic receptors that modulate norepinephrine release from adrenergic nerve endings (3, 9, 14, 19). Imamura et al. (14) reported that, in the heart, H₃ receptors decreased norepinephrine release in pathophysiological conditions associated with enhanced adrenergic activity, such as acute myocardial ischemia. The presence of modulatory H₃ receptors on adrenergic nerve terminals in the heart suggests their possible activation by an endogenous ligand, which is presumably histamine.

In animal models, sympathetic nerve stimulation has been shown to elicit a frequency-dependent release of cardiac histamine (9). Under normal adrenergic activity, histamine release appears to be too low to inhibit norepinephrine exocytosis (3, 9). In those instances, cardiac H₃ receptors are quiescent, and the presence of the histamine H₃ blocker thioperamide maleate (TM) fails to increase cardiac norepinephrine release. On the other hand, the H₃-receptor agonist (R)-α-methylhistamine (RAMH) causes an inhibition of the adrenergic inotropic response under normal conditions.

However, under pathological conditions, such as myocardial ischemia, Imamura et al. (14) showed that there was a 3.5-fold increase in cardiac histamine release compared with pres ischemic conditions and that H₃ receptors were fully activated by an endogenous ligand. Under such conditions, RAMH did not modify norepinephrine exocytosis, whereas TM doubled norepinephrine release. In association with blockade of cardiac H₃ activation by TM, augmentation of the adrenergic chronotropic response was observed in their ischemic model.

In sepsis, there is intense adrenergic stimulation as cardiovascular collapse develops over the course of the illness. This may lead to activation of cardiac H₃ receptors by endogenous ligand and contribute to cardiovascular collapse in sepsis. Under such circumstances, H₃-receptor blockade may restore cardiac adrenergic responses in sepsis and may lead to an improvement in cardiovascular function. In the present study we determined whether H₃ receptors played a role in the modulation of cardiac function in experimental Escherichia coli sepsis.

METHODS

In vivo protocols. The present study was approved by the Central Animal Care Committee at the University of Manitoba. In initial experiments the effect of H₃ blockade on hemodynamics was examined in four groups of dogs (hemodynamic study). The description of the sepsis model is given below. These groups included an H₃-blocker–sepsis group (n = 8), in which after 4 h of E. coli infusion, an H₃-blocking agent [TM at 2 mg/kg (n = 6) or clobenpropit at 0.6 mg/kg (n = 2) mixed in 250 ml of 5% dextrose in water (D₅W)] was administered over 30 min; a sepsis group (n = 8), in which after 4 h of E. coli infusion, placebo treatment (250 ml of D₅W) was administered over 30 min; an H₃-blocker group (n = 6), in which an H₃-blocking agent [TM at 2 mg/kg (n = 4) or clobenpropit at 0.6 mg/kg (n = 2) mixed in 250 ml of D₅W] was given after 4 h in nonseptic dogs; and a control group, in which placebo (250 ml of D₅W) was administered after 4 h in nonseptic dogs (n = 7). Because the findings obtained with both H₃-receptor blockers (i.e., TM and clobenpropit) were the same, the results were averaged as a single group. Clobenpropit was used in the subsequent experiments, because it has a longer duration of action than TM (18; see discussion).

In the hemodynamic study, measurements (see below) were obtained at presepsis (baseline); after 4 h of sepsis (4 h), during which hypotension occurs in this model (8); immediately after treatment or placebo was administered; and then at 0.5, 1, and 2 h after treatment.

Because hemodynamic differences were found between the H₃-blocker–sepsis and sepsis groups, a supplementary in vivo protocol (cardiac mechanics study) was performed in separate dogs in these groups, in which left ventricular (LV) contractil-

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ity was also measured (n = 4). In the H$_3$-blocker–sepsis group, two dogs received TM and two received clobenpropit. In the cardiac mechanics study, sonomicrometric techniques were used to determine LV volumes (see below). The sequence of measurements in this supplementary study was the same as that used in the hemodynamic study, except measurements were not obtained after the 0.5-h posttreatment period, since changes occurred early after H$_3$-receptor treatment in the hemodynamic study.

Preparation and measurements. In the sepsis groups, infection was induced by intravenous infusion of $10^{10}$ colony-forming units of live E. coli (designated type 0111:B4) (8). The bacteria were suspended in normal saline solution, which was given over 0.5 h. A constant infusion of $5 \times 10^9$ colony-forming units/h of E. coli was then maintained for the remainder of the experiment. In the nonsepsis groups the same amount of normal saline solution was given over this period.

In the hemodynamic and cardiac mechanics studies, the animals (20–30 kg) were anesthetized with thiopental so-

...sion, except measure-

...nts were obtained after the 0.5-h posttreatment period,

...as that used in the hemodynamic study, except measure-

...and the percent decrease in adrenergic activity was deter-

...A carotid artery incision was made, and a high-fidelity

...and the percent decrease in adrenergic activity was deter-

...Mongrel dogs (3–10 kg) were anesthetized with pentobarbi-

...tissue volume tracing to define end-diastolic dimen-

...arithmetic parameters unchanged. After 24 such pulses, the pulse width was reduced to 2 ms to restore control responses. The increase in tension seen with sympathetic nerve stimulation was calculated as percent increase from basal twitch amplitude.

...one set of experiments it was determined that, during field stimulation, propranolol (10$^{-5}$ M) completely abolished the adrenergic response (n = 5), whereas atropine had no effect. In another set of experiments the histamine H$_3$ agonist RAMH was added to the muscle bath at 0.01, 0.1, and 1 µM, and the percent decrease in adrenergic activity was determined in normal trabeculae (n = 5) (3, 14, 19). This was...
followed by the addition to the bath of the H3-receptor blocker clobenpropit (18) at 0.01, 0.1, and 1 µM, and the extent to which sympathetic stimulation was restored was assessed. As previously indicated, in sepsis the presence of modulatory H3 receptors on adrenergic nerve terminals in the heart would imply a possible action by an endogenous ligand that may be found in septic plasma. Accordingly, the effect of septic plasma fraction (<30,000 mol wt, see below) on the adrenergic response was examined in the trabecular preparation (n = 4). This effect was compared with that obtained when a nonseptic plasma fraction (<30,000 mol wt) was added to the trabecular bath (n = 4). Separate trabeculae were used when pre- and postsepsis plasma fractions were compared, but the trabeculae were obtained from the same donor dog. In this experiment, 0.5 ml of nonseptic and septic plasma fractions (<30,000 mol wt) were placed into respective organ baths, and the effect of clobenpropit (0, 0.001, 0.01, 0.1, and 1 µM) on modulation of the adrenergic response was ascertained.

Furthermore, the <30,000-mol wt fraction was chosen on the basis of previous studies which suggested that this plasma fraction contained a substance that contributed to cardiac depression in sepsis (8, 17; see DISCUSSION). A plasma fraction, <30,000 mol wt was obtained by pore filtration techniques, in which presepsis and then postsepsis plasma samples (30 ml) were passed through a 30,000-mol wt filter (Amicon) (8, 17).

In five other experiments the effect of increasing concentrations of histamine on basal isometric tension and sympathetic stimulation was determined in the in vitro preparation. The purpose was to determine whether histamine H3, H2, and H1 receptors are activated at different histamine concentrations (10, 29) and to determine whether the specific histamine H3 effect could indeed be attenuated by an H3-receptor blocker. The effect of histamine at 10^{-11}–10^{-3} M on basal twitch amplitude and the adrenergic response were determined.

Statistics. When multiple comparisons were obtained, the analyses consisted of one- and two-way ANOVA for repeated measures and Student-Newman-Keuls multiple-comparison test. When two comparisons were obtained, paired or unpaired t-tests were used in the appropriate circumstances. In the hemodynamic and cardiac mechanics studies the respective conditions between groups were compared by two-way ANOVA for two repeated measures (factor A, different treatment groups; factor B, different time periods), in which the interaction between the two factors was assessed. In this analysis, significance in the interaction term controls for experiment-wise error and repeated measurements (25). If a significant interaction was present, then the treatments behaved differently over time. In that case, a Student-Newman-Keuls multiple-range test was used to determine at which specific time periods a difference among groups was present. The results are expressed as means ± SD.

RESULTS

In the hemodynamic study, BP measured at 4 h fell (Fig. 1) by approximately one-half in both sepsis groups compared with baseline measurements. In the H3-blocker–sepsis group, BP immediately increased posttreatment compared with treatment. In contrast, in the placebo-treated sepsis group, BP did not increase between the 4-h period and posttreatment and continued to fall over the remainder of the study. By two-way ANOVA, the different findings in BP observed between the treated and untreated sepsis groups were statistically significant (i.e., significant interaction was present) over most of the posttreatment period. In contrast, there was no effect of the H3 blocker on BP in the nonsepsis group.

During treatment the immediate increase in BP observed in the H3-blocker–sepsis group was due to an
increase in CO (Fig. 1), since SVR did not change with treatment (Fig. 1; see below). Furthermore, CO remained higher than values found in the sepsis group over the remaining 2-h interval. The different findings in CO over time between the sepsis groups were statistically significant by two-way ANOVA. No changes in CO were observed with H₃-receptor blocker treatment in the nonsepsis group. Because HR was unchanged with H₃-blocker treatment in sepsis and nonsepsis groups, the changes in SV followed those in CO (Table 1).

In the sepsis and nonsepsis groups, H₃-receptor blocker therapy was associated with an increase in Pwp posttreatment (Table 1). In the H₃-blocker–sepsis group, this increase occurred immediately, but by 0.5 and 1 h posttreatment, Pwp values were not different in the two sepsis groups. In contrast, in the nonsepsis group, after H₃-blocker treatment, Pwp remained higher than corresponding values in the time-control group over the remainder of the study.

The changes in SVR are shown in Fig. 1. Compared with baseline measurements, SVR in the sepsis groups fell at 4 h and then remained unchanged for the duration of the study. There was no effect of the H₃-receptor blocker on SVR in the nonsepsis or the sepsis group.

At baseline, histamine concentrations (Table 2) were of intermediate magnitude compared with their respective companion groups. Whereas histamine concentrations fell in the time-control group at 4 h and then remained unchanged, there was no effect of the H₃-receptor blocker on SVR in the nonsepsis or the sepsis group.

In the cardiac mechanics study, sonomicrometric techniques were used to determine whether the higher CO observed with treatment in the H₃-blocker–sepsis group reflected an increase in LV contractility. PRSWR was used to assess LV systolic performance in which
LVEDV vs. SW coordinates were examined over a similar range of LVEDV between conditions; linear regression analysis was used to determine whether there was a change in slope between conditions.

In the nontreated dog, (Fig. 2A), after 4 h of sepsis, there was a shift in the relationship downward and to the right compared with the baseline relationship and no change in the relationship when placebo was administered. In contrast, in the H3-blocker–sepsis dog (Fig. 2B), H3-receptor blockade caused an improvement in LV contractility posttreatment compared with the untreated sepsis dog. The mean slopes are shown in Table 3. There were no changes in the intercepts observed in the H3-blocker–sepsis group (21 ± 6, 15 ± 13, 23 ± 5, and 23 ± 5 ml) or in the sepsis group (21 ± 12, 18 ± 9, 13 ± 7, and 15 ± 5 ml) over the four measurement periods, although there was wide variability in the individual dogs, which accounts for the apparent changes in intercepts in Fig. 2. Moreover, in the linear regression analysis, $R^2 > 0.92$ in all experiments.

In vitro experiments. An example of the increase in isometric contraction in response to field stimulation observed in the trabecular preparation is shown in Fig. 3A. The mean increase in adrenergic response was 51 ± 31% ($n = 6$). The experiment usually lasted 1–1.5 h, and, although basal tension slightly decreased over the course of the experiment ($n = 4, 0.41 \pm 0.1$ g at beginning to 0.31 g at 90 min, $P < 0.05$ vs. beginning), there was no effect of time on the percent adrenergic response, which changed <2% over the 1.5-h period. In Fig. 3B, propranolol completely blocked the adrenergic response.

The addition of the H3 agonist (RAMH) to the in vitro preparation caused a decline in the adrenergic response that was significant at 1 µM (Fig. 4). The further addition of the H3 blocker clobenpropit to the bath in the presence of RAMH (1 µM) totally reversed the decline in the adrenergic response at 1 µM. In other experiments, clobenpropit alone (1 nM–1 µM) did not

![Fig. 2. Stroke work plotted against left ventricular end-diastolic volume for different measurement conditions in cardiac mechanics study. A: dog in sepsis group; after 4 h of sepsis, relationship was shifted downward and to the right compared with baseline, and there was no response to placebo treatment. B: dog in H3-blocker–sepsis group; treatment was associated with a return in slope to presepsis value.](image)

![Fig. 3. A: isometric tension plotted against time in in vitro trabecular preparation. Adrenergic response is indicated by interval between arrows. Small dip in isometric tension at beginning of stimulation and slight increase in tension immediately after stimulation are believed to represent effect of synchronization as described by Blinks (1). This may be due to abnormal conduction of action potential when sympathetic stimulation is initially applied and then stopped (see Fig. 6). In B, an increase in adrenergic response could be observed before propranolol ($10^{-5}$ M) was added to bath (●), but not after this treatment.](image)

**Table 3. Slope of PRSWR in the cardiac mechanics study**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>4 h</th>
<th>Treatment</th>
<th>0.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3-blocker-sepsis</td>
<td>90 ± 28*</td>
<td>47 ± 30</td>
<td>97 ± 30*</td>
<td>107 ± 18†</td>
</tr>
<tr>
<td>Sepsis</td>
<td>96 ± 46</td>
<td>72 ± 12</td>
<td>61 ± 10</td>
<td>63 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SD in mmHg; $n = 4$ dogs. PRSWR, preload recruitable stroke-work relationship. *$P < 0.05$ vs. 4 h within a group by 1-way repeated-measures ANOVA and Student-Newman-Keuls test. †$P < 0.05$ vs. sepsis group (by 2-way ANOVA and Student-Newman-Keuls test).
affect the adrenergic response, which remained at 37 ± 29% (SD).

In the presence of the nonseptic plasma fraction (<30,000 mol wt), H3-receptor blockade did not change the adrenergic response compared with the pretreatment value (Fig. 5). In contrast, in the presence of the septic plasma fraction, the addition of the H3 blocker to the trabecular preparation increased the adrenergic response (Figs. 5 and 6). Moreover, as was observed in our time-control muscles, there was a comparable decline in basal tension over time that was not different in conditions of septic and nonseptic plasma fractions and occurred when the higher doses of clobenpropit were studied (i.e., 100 nM and 1 µM).

The effect of various concentrations of histamine on the adrenergic response and basal twitch amplitude is shown in Fig. 7. At the low concentrations (10^{-11}–10^{-7} M), histamine preferentially inhibited the adrenergic response and had no effect on basal twitch tension. Before histamine was added to the bath, the increase in adrenergic response was 40 ± 33%, decreased to 20 ± 10% at 10^{-11} M, further declined to 10 ± 15% with 10^{-7} M histamine, and finally measured near zero with 10^{-5} M histamine. This inhibition could be blocked by clobenpropit or TM. In contrast, at higher concentrations (10^{-5} M), histamine H1 receptors (which caused depression) and H2 receptors (which increased inotropy) were activated and basal twitch amplitude was altered (10). Furthermore, H1 and H2 effects could be modulated by pyrilamine maleate (10^{-5} M) and cimetidine metiamide (10^{-5} M), respectively, but not by H3 blockers.

DISCUSSION

The purpose of this study was to assess the relevance of histamine H3 receptors to cardiovascular function in sepsis. Imamura et al. (14) found that histamine H3 receptors inhibited norepinephrine release under conditions of enhanced adrenergic activity in an experimental model of ischemia. Because enhancement of adren-
that activation of peripheral H3 receptors resulted in a decrease in tone in arterial resistance vessels. How-ever, we found that the higher BP found in the H3-blocker–sepsis group was due to an increase in CO, since SVR did not change with blockade. MclLeod et al. (19) found that activation of peripheral H3 receptors resulted in lower basal SVR in a guinea pig preparation. This suggested that activation of H3 receptors may cause a decrease in tone in arterial resistance vessels. However, we could not reverse the lower SVR found in sepsis with H3-receptor blockade. This may indicate that the reduction in peripheral resistance observed in sepsis is not caused by activation of H3 receptors in a major way. Other mediators released during sepsis, related to products of the prostaglandin pathway (16), to vascular nitric oxide production (13), or to other pathways, may account for the lower SVR found in sepsis.

In the H3-blocker–sepsis study, although preload (Pwp) was not different from that found in the sepsis group over most of the measurement intervals, Pwp increased transiently after treatment compared with the 4-h value (Table 1). Histamine H3 receptors have been identified in splanchnic tissues, such as guinea pig ileum and duodenum (11, 23). H3 blockade may have reduced vascular compliance in the splanchic circulation, leading to an increase in venous return and to a higher Pwp after treatment. Thus, to some extent, an increase in preload may have contributed to the higher CO found in the H3-blocker–sepsis group, but whether contractility also increased was not clear. In the cardiac mechanics study, as determined by PRSWR (6), we found that, compared with the 4-h measurement, LV contractility increased after H3-receptor blockade.

The presence of modulatory H3 receptors on adrenergic nerve terminals in the heart infers their possible activation by an endogenous ligand, possibly histamine (3, 9, 14). Gross et al. (9) previously showed that, under conditions of sympathetic nerve stimulation, there was a frequency-dependent release of cardiac histamine, whereas others have shown that ischemia promotes the release of cardiac histamine in experimental models (28).

In the hemodynamic study we measured histamine concentrations of samples taken from the femoral artery. In the H3-blocker–sepsis group, concentrations increased ~2.5 times over the course of the study compared with baseline (P < 0.11) and were significantly different from the time-control group. In another study, Brackett et al. (2) found more consistent increases in plasma histamine concentrations in an endotoxin model where plasma histamine concentrations increased from 10 to 30 ng/ml over 4 h of sepsis, whereas there was no change in the control group. The present study shows that the concentrations of histamine required for H3-receptor activation appear to be small (Fig. 7). This activation could therefore be produced by concentrations as low as 10⁻¹¹ M, which would be outside the sensitivity of our assay.

Furthermore, whereas histamine H3 activation occurs without altering basal tension, histamine H2- and H2-receptor activation affect inotropy, causing a decrease and an increase in contractility, respectively (Fig. 7) (10). Basal depression in myocardial function has been shown in human subjects and animal models (7, 21, 22) and has been related to (among others) the release of cytokines and the formation of an inducible nitric oxide synthase (4, 8, 24). In the in vitro study we used the <30,000-mol wt plasma fraction to represent septic plasma, because we previously showed that it contains a factor that causes a depression in basal contraction in sepsis (17). Histamine would also be found in this fraction. However, the present study shows that the histamine concentrations would not be large enough to effect basal contractility in this model.

In terms of the effect of histamine H3 activation on norepinephrine release, Imamura et al. (14) found that the detectable norepinephrine overflow during sympathetic stimulation was relatively small in an isolated heart preparation and that the overflow was maximal at 60 s of stimulation (40 pmol/g) and then decreased to 30 pmol/g during H3 activation. In an endotoxin model of sepsis, Brackett et al. (2) showed that plasma norepinephrine concentrations increased from 250 pg/ml at baseline to 1,500 pg/ml over a 4-h interval, in which this increase would come from all sources, adrenal and extra-adrenal. Because in sepsis the total increase in...
plasma norepinephrine concentrations may be large compared with the amount due to sympathetic neural overflow per se, in the design of the in vitro study, rather than to directly measure norepinephrine release, our approach was to examine the effect of H₃-receptor blockade on improving hemodynamics, which was the important end point of the study.

In the present study we used TM and clobenpropit as H₃-receptor blockers. We initially used TM on the basis of the work of McLeod et al. (19). Subsequent work showed that clobenpropit may have a longer period of action and higher potency, so we switched to TM (18). Recognize, however, that it was not the purpose of this study to obtain a dose-response relationship of the different agents, but only to determine whether histamine H₃ activation was present in sepsis. Both agents showed similar effects in our model.

In the present study, moreover, we used an in vitro ventricular preparation to corroborate our in vivo findings. However, these results must be interpreted cautiously. In the in vitro preparation, field stimulation was used to produce sympathetic stimulation, but this approach is a very unphysiological way of causing norepinephrine release. A more physiological approach would be to use an innervated preparation in which the sympathetic nerves could be directly stimulated. Furthermore, the results showed that propranolol blocked the increase in isometric tension observed during field stimulation, and the conclusion was that the sympathetic nervous system caused the changes in muscle tension. However, propranolol has numerous effects, including direct membrane stabilization (12), and therefore it is uncertain that sympathetic stimulation accounted for the entire contractile effect during field stimulation.

In summary, in an in vivo model of sepsis, H₃-receptor blockade was associated with an improvement in hemodynamics, which reflected at least in part an increase in LV contractility. In an in vitro ventricular preparation the results showed that a substance in the septic plasma fraction caused an inhibition of the cardiac adrenergic response that was amenable to H₃-receptor blockade. Although the present study favors the idea that histamine H₃ blockade augments norepinephrine release from adrenergic nerve endings, it is also possible that histamine H₃ blockers may improve hemodynamics in sepsis by as yet undefined mechanisms. Furthermore, it is important to recognize that the findings obtained in this animal model and the in vitro preparation may not reflect those in human disease and that the animals were studied under anesthesia, which may also have affected the results. Within the context of these limitations, however, we conclude that activation of H₃ receptors may contribute to cardiovascular collapse in sepsis.

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