Activation of histamine H3 receptors inhibits renal noradrenergic neurotransmission in anesthetized dogs

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Yamasaki, Tomoyuki, Isao Tamai, and Yasuo Matsumura. Activation of histamine H3 receptors inhibits renal noradrenergic neurotransmission in anesthetized dogs. Am J Physiol Regulatory Integrative Comp Physiol 280: R1450–R1456, 2001.—To investigate the possible involvement of histamine H3 receptors in renal noradrenergic neurotransmission, effects of (R)alpha-methylhistamine (R-HA), a selective H3-receptor agonist, and thioperamide (Thiop), a selective H3-receptor antagonist, on renal nerve stimulation (RNS)-induced changes in renal function and norepinephrine (NE) overflow in anesthetized dogs were examined. RNS (0.5–2.0 Hz) produced significant decreases in urine flow and urinary sodium excretion and increases in NE overflow rate (NEOR), without affecting renal hemodynamics. When R-HA (1 μg·kg⁻¹·min⁻¹) was infused intravenously, mean arterial pressure and heart rate were significantly decreased, and there was a tendency to reduce basal values of urine flow and urinary sodium excretion. During R-HA infusion, RNS-induced antidiuretic action and increases in NEOR were markedly attenuated. Thiop infusion (5 μg·kg⁻¹·min⁻¹) did not affect basal hemodynamic and excretory parameters. Thiop infusion caused RNS-induced antidiuretic action and increases in NEOR similar to the basal condition. When R-HA was administered concomitantly with Thiop infusion, R-HA failed to attenuate the RNS-induced antidiuretic action and increases in NEOR. However, in the presence of pyrilamine (a selective H1-receptor antagonist) or cimetidine (a selective H2-receptor antagonist) infusion, R-HA attenuated the RNS-induced actions, similarly to the case without these antagonists. Thus functional histamine H3 receptors, possibly located on renal noradrenergic nerve endings, may play the role of inhibitory modulators of renal noradrenergic neurotransmission.

Effects on cardiac functions of (R)alpha-methylhistamine (R-HA), a selective agonist of H3 receptor, have been reported. McLeod at al. (24) found that R-HA decreased blood pressure with ensuing tachycardiac and bradycardiac effects in anesthetized guinea pigs and related rabbits, respectively, hence indicating a species difference in cardiovascular responses. R-HA attenuates inotropic and chronotropic responses of isolated guinea pig atria to transmural stimulation of adrenergic nerve endings (8). In addition, this attenuation is associated with a marked decrease in endogenous NE release (8). Similar inhibitory effects of R-HA on NE release have been observed in sympathetic nerve endings isolated from human atria (16). Mazenot et al. (23) reported that R-HA inhibits NE release and related hemodynamic effects induced by the electrical stimulation of cardiac nerve endings in anesthetized dogs. These findings strongly suggest the important role of presynaptic H3 receptors as inhibitory modulators of cardiac noradrenergic neurotransmission.

The functional role of H3 receptors in the kidney has remained obscure. Renal sympathetic nerve activity is closely involved in regulatory mechanisms of renal hemodynamics and excretory responses. NE released from nerve endings leads to renal vasoconstriction and an increased tubular reabsorption to diminish renal hemodynamics, urine flow (UF), and urinary excretion of sodium (UNaV), respectively (21, 22, 32). We investigated if activation of H3 receptors in the kidney would modulate the release of NE from the sympathetic nerve endings, and for this we examined effects of R-HA and thioperamide (Thiop), a selective H3-receptor antagonist, on renal nerve stimulation (RNS)-induced renal actions and NE overflow in anesthetized dogs. We now report here the first evidence for functional H3 receptors as inhibitory modulators of renal noradrenergic neurotransmission in dogs.

MATERIALS AND METHODS

Animal preparation. Adult male beagle dogs weighing 9.5–13.5 kg were used. These dogs were anesthetized with pentobarbital sodium (30 mg/kg iv) and were given maintenance doses as needed. These dogs were placed on a heated surgical
table that maintained the rectal temperature between 37 and 38°C. After tracheal intubation of the animals, respiration was supported by artificial ventilation with room air, using a Harvard respirator. Polyethylene catheters were placed in the right brachial artery and vein for arterial blood sampling and for infusion of saline or drug solution containing 0.45% inulin, respectively. Mean arterial blood pressure (MAP) and heart rate (HR) were monitored using a pressure transducer (Nihon Kohden, AP601G, Tokyo, Japan) connected to a polyethylene catheter placed in the abdominal aorta via the right femoral artery. The left kidney was exposed retroperitoneally through a flank incision, and the renal artery was isolated from surrounding tissues. All visible nerve fibers along the renal artery were isolated, ligated, and cut. For RNS, the distal cut portion was placed on bipolar platinum electrodes connected to an electric stimulator (Nihon Kohden, SEN-7103). An electromagnetic flow probe (2.0–3.5 mm in diameter) connected to a square-wave flowmeter (Nihon Kohden, MFV-2100) was attached around the left renal artery to continuously measure renal blood flow (RBF). A curved 18-gauge needle connected to polyethylene tubing was inserted into the left renal vein for venous blood sampling. The left ureter was then cannulated for urine collection. After completing the surgical procedures, a priming dose of inulin (20 mg/kg) was given, followed by infusion of 0.9% saline containing 0.45% inulin for purposes of measuring the glomerular filtration rate (GFR), at a rate of 2.0 ml/min. The MAP, HR, and RBF were continuously recorded on a polygraph (Nihon Kohden, RM6000G). About 2 h were allowed for stabilization.

Experimental protocol (experiment 1: effects of R-HA on RNS-induced renal actions). Two RNS experiments were done on each of seven dogs. Each experiment consisted of a 10-min control period and a 10-min RNS period. Blood samples (3.0 ml) were taken at 5 min in the control period and at 1 and 9 min in the RNS period from the right brachial artery and left renal vein, respectively. After the systemic arterial hematocrit was measured by the microcapillary method, plasma was immediately separated by centrifugation. Urine samples were collected during the latter 5 min in each period. Hemodynamic parameters such as MAP, HR, and RBF were determined at the midpoint of each period.

During the first RNS experiment (0.5–2.0 Hz, duration 1.0 ms, and supramaximal voltage 10–25 V), saline was infused. About 30 min after the first RNS experiment was terminated, intravenous infusion of R-HA (1 μg·kg⁻¹·min⁻¹) was started. After 30 min, the second RNS experiment was done under conditions of drug infusion in the same manner as the first experiment. In this study, RNS was done at a low frequency that has no influence on systemic and renal hemodynamics (12).

Experimental protocol (experiment 2: effects of R-HA on RNS-induced renal actions in the presence of Thiop). Three RNS experiments were done in six dogs. After equilibration, the first RNS experiment was done during saline infusion, in the same manner as for experiment 1. About 30 min after the first experiment was terminated, the intravenous infusion of Thiop (5 μg·kg⁻¹·min⁻¹) was started. After 30 min, the second RNS experiment was done in the presence of Thiop. The third experiment was done during the simultaneous infusion of R-HA (1 μg·kg⁻¹·min⁻¹) with Thiop (5 μg·kg⁻¹·min⁻¹). The dose of Thiop was determined based on inhibitory effects of Thiop on R-HA-induced changes in systemic hemodynamics. In separate experiments, pyrilamine (5 μg·kg⁻¹·min⁻¹, a selective H₁-receptor antagonist) (13) or cimetidine (100 μg·kg⁻¹·min⁻¹, a selective H₂-receptor antagonist) (13) was used instead of Thiop.

Analytic procedures. GFR was estimated as based on inulin clearance. The urine and plasma inulin levels were measured spectrophotometrically (Hitachi, 650–660, Hitachinaka, Japan) according to Vurek and Pegram (33). Urine and plasma sodium concentrations were determined using a flame photometer (Hitachi, 205D). The plasma NE concentration was measured using high performance liquid chromatography and an amperometric detector (ECD-100, Eicom, Kyoto, Japan), as reported (12). The NE overflow rate (NEOR) was calculated by NEOR (pg·g⁻¹·min⁻¹) = (NEV−NEao)RPF, where RPF is the renal plasma flow (μl·kg⁻¹·min⁻¹), NEV is the renal venous plasma NE concentration (pg/ml), and NEao is the renal arterial plasma NE concentration (pg/ml). NEOR served for measurement of renal NE spillover, which reflects the renal sympathetic nervous activity (9).

Drugs. R-HA and Thiop were purchased from Sigma Chemical (St. Louis, MO), and all other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan).

Statistical analysis. All data are expressed as means ± SE. For statistical analyses, we used paired or unpaired Student’s t-test for two-sample comparisons. When comparing normalized data in three groups, we used Kruskal-Wallis nonparametric ANOVA test followed by Dunn’s multiple comparison test. For all comparisons, differences were considered to have statistical significance at P < 0.05.

RESULTS

Effects of R-HA on RNS-induced renal actions (experiment 1). RNS at 0.5–2.0 Hz decreased UF, UNaV, and fractional excretion of sodium (FENa) by 64, 65, and 62% from each control values of 22.4 ± 31%, 4.49 ± 3.41%, respectively, without affecting systemic and renal hemodynamics (Fig. 1, Table 1). When R-HA (1 μg·kg⁻¹·min⁻¹) was intravenously infused, basal values of MAP and HR were significantly decreased (Table 1). In addition, there was a tendency to reduce basal values of UF, UNaV, and FENa, although observed changes were not statistically significant (Fig. 1). During R-HA infusion, RNS-induced decreases in UF, UNaV, and FENa were significantly attenuated, and observed changes were −37, −30, and −31%, respectively (Fig. 1). No significant changes in systemic and renal hemodynamics were observed in RNS during the R-HA infusion (Table 1).

Effects of R-HA on RNS-induced increase in NEOR (experiment 1). RNS during saline infusion significantly increased NEOR from a control value of −573.6 ± 165.4 to 155.1 ± 275.7 and 148.8 ± 259.3 pg·g⁻¹·min⁻¹ at 1 and 9 min after the start of RNS, respectively. In the following results, RNS-induced increases in NEOR from control are indicated as ΔNEOR to clarify changes in NEOR induced by the RNS. The intravenous infusion of R-HA significantly attenuated increases in ΔNEOR during RNS (from 728.7 ± 178.1 and 722.4 ± 194.6 to 152.0 ± 28.4 and 315.7 ± 61.2 pg·g⁻¹·min⁻¹ at 1 and 9 min after the start of RNS, respectively; Fig. 2).

Effects of R-HA on RNS-induced renal actions in the presence of Thiop (experiment 2). Intravenous infusion of Thiop (5 μg·g⁻¹·min⁻¹) had no apparent effect on basal systemic and renal hemodynamics, and excretory...
responses (Table 2, Fig. 3). RNS produced no significant effects on systemic and renal hemodynamics in the presence or absence of Thiop (Table 2). The RNS-induced attenuation of excretory responses during saline infusion (UF, UNaV, and FENa decreased by 49%, 41%, and 41% from control values of 34.6 ± 5.1 μl·g⁻¹·min⁻¹, 7.00 ± 1.00 μeq·g⁻¹·min⁻¹, and 5.79 ± 0.68%, respectively) was not significantly changed by Thiop (Fig. 3). When R-HA was administered in the presence of Thiop infusion, R-HA-induced decreasing actions on MAP, HR, RBF, and UF were not observed (Table 2, Fig. 3). During the simultaneous infusion of R-HA and Thiop, systemic and renal hemodynamics were not significantly changed by RNS (Table 2). As shown in Fig. 3, when R-HA was administered in the presence of Thiop infusion, R-HA failed to attenuate the RNS-induced antidiuretic and antinatriuretic actions.

**Effects of R-HA on RNS-induced increase in NEOR in the presence of Thiop (experiment 2).** As shown in Fig. 4, RNS-induced increases in ΔNEOR during saline infusion were not affected by Thiop infusion. In addition, when R-HA was infused simultaneously with Thiop, the RNS-induced increases in ΔNEOR were similar to those observed with saline infusion (Fig. 4).

**Effects of R-HA on RNS-induced renal actions and increase in NEOR in the presence of pyrilamine or cimetidine (experiment 2).** To verify the receptor specificity of R-HA-induced inhibitory effects on renal actions and NE overflow in response to the RNS, effects of R-HA were examined in the absence or presence of pyrilamine (a selective H₁-receptor antagonist) or cimetidine (a selective H₂-receptor antagonist). Intravenous infusion of pyrilamine (5 μg·kg⁻¹·min⁻¹) had no apparent effect on basal systemic and renal hemodynamics or excretory responses. During pyrilamine in-

**Table 1. Effects of R-HA on RNS-induced changes in systemic and renal hemodynamics in anesthetized dogs**

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, ml·g⁻¹·min⁻¹</th>
<th>RVR, mmHg·ml⁻¹·g⁻¹·min⁻¹</th>
<th>GFR, ml·g⁻¹·min⁻¹</th>
<th>FF, %</th>
</tr>
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<tr>
<td><strong>Saline infusion</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>154.7 ± 2.3</td>
<td>198.3 ± 8.7</td>
<td>3.94 ± 0.38</td>
<td>41.3 ± 3.92</td>
<td>0.91 ± 0.06</td>
<td>42.3 ± 2.23</td>
</tr>
<tr>
<td>%Change</td>
<td>0.0 ± 0.5</td>
<td>2.8 ± 1.7</td>
<td>-0.45 ± 1.3</td>
<td>1.1 ± 1.4</td>
<td>-10.36 ± 5.3</td>
<td>-7.94 ± 4.3</td>
</tr>
<tr>
<td><strong>R-HA infusion</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>137.9 ± 6.3*</td>
<td>180.7 ± 9.1†</td>
<td>3.79 ± 0.42</td>
<td>39.5 ± 4.69</td>
<td>0.92 ± 0.08</td>
<td>41.6 ± 2.84</td>
</tr>
<tr>
<td>%Change</td>
<td>0.7 ± 3.5</td>
<td>1.5 ± 0.5</td>
<td>1.91 ± 2.7</td>
<td>-0.77 ± 2.5</td>
<td>-3.93 ± 7.7</td>
<td>-6.00 ± 6.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of 7 dogs. *P < 0.05, †P < 0.01, compared with control value during saline infusion. MAP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction; RNS, renal nerve stimulation; R-HA, (R)α-methylhistamine.
Effects of Thiop and R-HA on RNS-induced changes in systemic and renal hemodynamics

Table 2. Effects of Thiop and R-HA on RNS-induced changes in systemic and renal hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, ml g⁻¹ min⁻¹</th>
<th>RVR, mmHg ml g⁻¹ min⁻¹</th>
<th>GFR, ml g⁻¹ min⁻¹</th>
<th>FF, %</th>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>146.4 ± 6.3</td>
<td>159.3 ± 6.2</td>
<td>3.2 ± 0.2</td>
<td>46.7 ± 3.4</td>
<td>0.80 ± 0.07</td>
<td>46.8 ± 1.8</td>
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<tr>
<td>RNS</td>
<td>147.8 ± 6.3</td>
<td>159.8 ± 6.4</td>
<td>3.1 ± 0.2</td>
<td>48.0 ± 3.9</td>
<td>0.80 ± 0.05</td>
<td>46.5 ± 2.5</td>
</tr>
<tr>
<td>%Change</td>
<td>0.7 ± 0.8</td>
<td>0.2 ± 0.5</td>
<td>−0.8 ± 1.0</td>
<td>2.2 ± 1.3</td>
<td>0.3 ± 3.6</td>
<td>0.7 ± 4.2</td>
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<tr>
<td>Thiop infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>148.0 ± 7.3</td>
<td>157.8 ± 7.0</td>
<td>3.3 ± 0.3</td>
<td>46.2 ± 4.3</td>
<td>0.86 ± 0.07</td>
<td>47.7 ± 2.6</td>
</tr>
<tr>
<td>RNS</td>
<td>148.5 ± 7.0</td>
<td>160.1 ± 8.2</td>
<td>3.2 ± 0.3</td>
<td>49.1 ± 5.0</td>
<td>0.85 ± 0.05</td>
<td>48.7 ± 2.9</td>
</tr>
<tr>
<td>%Change</td>
<td>0.4 ± 0.8</td>
<td>1.4 ± 0.5</td>
<td>−5.1 ± 1.6</td>
<td>5.8 ± 1.7</td>
<td>0.3 ± 6.3</td>
<td>2.1 ± 4.7</td>
</tr>
<tr>
<td>Thiop + R-HA infusion</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>144.17 ± 8.9</td>
<td>152.0 ± 10.4</td>
<td>3.3 ± 0.4</td>
<td>47.0 ± 5.6</td>
<td>0.82 ± 0.06</td>
<td>46.3 ± 3.1</td>
</tr>
<tr>
<td>RNS</td>
<td>146.33 ± 9.3</td>
<td>155.7 ± 11.0</td>
<td>3.1 ± 0.4</td>
<td>49.8 ± 5.9</td>
<td>0.76 ± 0.09</td>
<td>44.7 ± 3.9</td>
</tr>
<tr>
<td>%Change</td>
<td>0.4 ± 0.4</td>
<td>2.4 ± 1.1</td>
<td>−4.8 ± 2.9</td>
<td>6.0 ± 2.9</td>
<td>−6.7 ± 7.3</td>
<td>−3.2 ± 6.2</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of 6 dogs. Thiop, thioperamide.
events abolished by the simultaneous infusion of Thiop, thereby suggesting that R-HA-induced actions are mediated by the activation of H₃-receptor subtype. These results are in agreement with findings by McLeod et al. (24), who noted that R-HA-induced hypotensive and bradycardiac effects in guinea pigs were blocked by Thiop but not by H₁ antagonist chlorpheniramine or H₂ antagonist cimetidine. On the other hand, Thiop alone had no apparent effects on basal systemic hemodynamics. There are conflicting findings with respect to the cardiovascular effects of H₃ antagonists. In guinea pigs, rabbits, and rats, H₃ antagonists failed to induce significant cardiovascular effects (6, 24). In contrast, Mazenot et al. (23) noted vasopressor...
and tachycardiac effects in dogs in response to treatment with the H₃ antagonist SC-359, and they suggested that H₃ receptors are tonically functional and activated by endogenous histamine to regulate the cardiac function. The reason for these discrepancies is unclear, but there may be species differences (and/or differences in experimental conditions) in cardiovascular responses to H₃-receptor activation and inhibition, as suggested by MacLeod et al. (24).

There are reports indicating that a pathological condition leads to an increase in endogenous histamine levels and activation of H₃ receptors (11, 15). Imamura et al. (15) noted that in isolated guinea pig hearts exposed to ischemia-reperfusion, H₃ receptors were fully activated by increased endogenous histamine to suppress NE overflow from sympathetic nerves; therefore, Thiop markedly potentiated the ischemia-reperfusion-induced NE release, in contrast to the finding that Thiop had no effects on sympathetic nerve stimulation-induced NE overflow under physiological conditions. Thus, although there is information on the physiological and pathological roles of H₃ receptors in the heart, the functional roles of this receptor in the kidney have remained an open question. In our study, R-HA inhibited RNS-induced changes in renal function and NE overflow, and this attenuating effect of R-HA was antagonized by the selective H₃-receptor antagonist Thiop. However, basal renal function and RNS-induced renal actions were not affected by blockade of the H₃ receptor. We suggest that in the dog kidney under physiological conditions, histamine H₃ receptors are present and available for exogenously applied ligand to negatively modulate NE release, whereas endogenous histamine does not tonically activate H₃ receptor, as seen in the guinea pig heart (15). Whether endogenous histamine levels, under renal pathological conditions, are sufficiently elevated to activate H₃ receptors remains the subject of further study.

It is well acknowledged that NE release from sympathetic nerve endings is modulated by a presynaptic α₂-adrenergocceptor-mediated inhibitory mechanism. In the same experimental system using anesthetized dogs, intrarenal administration of yohimbine potentiated the RNS-induced renal vasoconstriction and NE overflow, thereby indicating that the presynaptic α₂-adrenergocceptor-mediated inhibitory mechanism exists in the dog kidney, which can be activated by endogenously released NE (14). In our study, RNS-induced renal actions and NE overflow were not enhanced by the administration of Thiop, thus suggesting that Thiop does not affect the presynaptic α₂-adrenergocceptor-mediated inhibitory mechanism.

By way of summary, histamine H₃-receptor activation by R-HA attenuated the RNS-induced antidiuretic action and NE overflow in anesthetized dogs, events abolished by treatment with Thiop, a selective H₃-receptor antagonist. We propose an important role for this receptor subtype as an inhibitory modulator of renal noradrenergic neurotransmission at the presynaptic level.

**Perspectives**

In various organs and vascular tissues, neurotransmitter release from sympathetic nerve endings is modulated by released NE itself and by various humoral factors through stimulation of their receptors located at presynaptic sites. Also, in the kidney there are facilitatory β₂-adrenergic and inhibitory α₂-adrenergic receptors. In addition, humoral factors such as angiotensin II, prostaglandin E₂, nitric oxide, and endothelin-1 have been demonstrated to function as a facilitatory or an inhibitory modulator of renal noradrenergic neurotransmission via prejunctional mechanisms, although the precise mechanism, including signaling pathways, remains to be elucidated (7, 20, 30, 31). In the present study, we reported the first evidence for functional histamine H₃ receptor as an inhibitory modulator of renal noradrenergic neurotransmission in dogs. Because H₃-receptor antagonist alone failed to enhance the RNS-induced NE overflow, physiological level of endogenous histamine does not seem to inhibit tonically the release of NE in the kidney. On the other hand, there is a possibility that the H₃ receptor-mediated action to negatively modulate NE release at renal sympathetic nerve endings may play an important role, if an endogenous histamine level is elevated under some pathological conditions. In the ischemic heart, histamine H₃ receptors appear to be fully activated by an increased endogenous histamine to suppress NE overflow from sympathetic nerves (15). Therefore, H₃ agonists may offer a novel therapeutic approach to myocardial ischemia, as suggested by Levi and Smith (18). Whether this view is applicable to the ischemic kidney remains the subject of further study.

We thank M. Ohara for language assistance.

**REFERENCES**