THE EFFECT OF BETA ADRENERGIC RECEPTOR BLOCKADE ON THE RENIN RESPONSE TO RESPIRATORY ACIDOSIS

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The effect of beta adrenergic blockade on the increase in plasma renin activity produced by acute respiratory acidosis was studied in chloralose anesthetized dogs. Sixteen mongrel dogs were given 4%, 8% and 12% CO₂ in room air, successively. Propranolol (2 mg/Kg) was given to 8 dogs prior to CO₂ inhalation. The other 8 dogs served as the control group. The response of elevated plasma renin activity during 4% and 8% CO₂ inhalation was not different between the control and propranolol groups. However, the increase of plasma renin activity in the control group was greater than that of the propranolol treated group during 12% CO₂ inhalation. It is suggested that activation of beta adrenergic receptors is not the sole factor in renin control during acute respiratory acidosis, although these receptors do mediate a significant fraction of the renin response to CO₂ inhalation.

Recently it was reported\(^1\) that plasma renin activity increased during acute respiratory acidosis and that the response was dependent on elevated PaCO₂ and/or decreased arterial pH rather than altered renal blood flow. It was hypothesized that the renin response had been a result of either sympathetic activation or the intrarenal vascular effects of elevated arterial PaCO₂, or a combination of these factors.

A variety of stimuli such as non-hypotensive hemorrhage\(^2\) assumption of the upright position and sodium deprivation\(^3\) provoke renin release by neural, tubular and/or humoral mechanisms. In acute respiratory acidosis, stimulation of central and peripheral chemoreceptors by elevated PaCO₂ or decreased pH has been shown to alter renal hemodynamics by the sympathetic nervous system\(^4,5\). Therefore, it was suggested that elevated PaCO₂ and/or decreased pH may elicit renin release by sympathetic influences on the renal vascular beds. However, Morita\(^6\) reported that renin production from the kidney was not blocked by the ganglionic blocker, hexamethonium, during inhalation of 5% and 15% CO₂ in dogs.

Winer et al. reported acute elevations of plasma renin activity following administration of diazoxide, ethacrynic acid and theophylline, and upon assumption of an upright position. Much evidence has accumulated which points to beta

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Key Words:
- Plasma renin activity
- Propranolol
- Arterial PaCO₂ and pH
- Renal blood flow
- Respiratory acidosis
- Beta adrenergic receptor
- Blood pressure
- Renal vascular resistance

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adrenergic receptor involvement during renin release. Michelakis and Winer have indicated that administration of the beta adrenergic antagonist propranolol to normal subjects blocked the acute rise in renin activity normally observed following a variety of stimuli. In other studies it has been shown that, in the dog, the renin response to epinephrine was blocked by beta adrenergic blockade but not by administration of alpha blocking agents. Although the mechanism for increased plasma renin activity during acute respiratory acidosis is unclear, we hypothesized that it may be mediated by beta adrenergic influences. The present study reports on work which studied the renin response to respiratory acidosis following beta receptor blockade.

MATERIALS AND METHODS

Experiments were conducted in 16 mongrel dogs (9.0–16.8 Kg) of both sexes on a normal sodium diet. All dogs were subjected to a similar protocol designed to induce reproducible levels of respiratory acidosis. Eight dogs acted as a control group while the other dogs were studied following propranolol administration (2 mg/Kg i.v.) prior to the initiation of acidosis. Anesthetic induction was initiated with sodium methohexital (sodium brevital 1% i.v.) and supplemented with an initial dose of alpha chloralose (100 mg/Kg, i.v.) prepared in 0.9% saline to a final concentration of 1%. Additional chloralose was administered (ca. 10 mg/30 min) as required to maintain a constant anesthetic plane. The animals were intubated and positive pressure ventilation was initiated with a Harvard Instruments Model 607 respirator. Alveolar ventilation was assured and atelectasis prevented by maintaining an end-expiratory alveolar pressure of 3–4 cm of water. Ventilation rate was set at 15 breaths per minute and tidal volume was adjusted so that under control conditions the end expiratory CO₂ was maintained at 5%. This provided a reproducible baseline for initial control studies. Percent CO₂ was measured on a calibrated rapid responding (100 msec) Beckman Instruments LB-2 respiratory gas analyzer. The signal from the gas analyzer was interfaced with a Beckman Instruments Type RM 6-channel inkwriting Dynograph and recorded continuously. A catheter (P.E. 204), advanced from the femoral vein to a location in the abdominal vena cava, was used for infusion of supplemental doses of anesthetic and for replacement of fluid volumes equal to those withdrawn during blood sampling.

An abdominal aortic catheter (P.E. 204) was inserted via a femoral artery for purposes of recording arterial blood pressure and for withdrawing arterial samples for analysis of pH, PaCO₂ and PaO₂. Samples were analyzed with an Instruments Laboratores Model 113-03 digital display blood gas analyzer. A Statham Instruments P23db pressure transducer measured arterial blood pressure which was continuously monitored as both a pulsatile and electronically averaged mean. The aortic arterial pulse served as an input to a Beckman Instruments Type 9857B Cardiotachometer for determination of heart rate. In each dog the left renal artery was exposed via a retroperitoneal flank approach and a noncannulating electromagnetic flow transducer was placed around the renal artery. Special care was taken to avoid damage to the renal nerve supply. Renal blood flow was measured with a Zepeda Instruments SW-3 square wave electromagnetic blood-flowmeter and continuously recorded as both pulsatile and electronically averaged mean. Flow transducers were calibrated in-vivo on a femoral artery with step flow controlled by a Harvard Instruments infusion-withdrawal pump.

RENIN ASSAY

Two ml blood samples were drawn in a 3 ml syringe containing 0.05 ml of 10% sodium EDTA to prevent clotting and to suppress angiotensinase activity. The samples were delivered into chilled glass tubes and placed in an ice bath for processing following completion of the experiment. Renin activity was determined by radioimmunoassay of angiotensin I using Zehr's modification of Haber's methods. Plasma was separated at 4°C and diluted with an equal volume of 0.05M sodium phosphate buffer (pH 7.4) containing 0.125% neomycin sulfate as a bacteriostatic agent. Ten microliters of 0.806M dimercaprol and 20 microliters of 0.340M 8-OH quinoline sulfate were added to each sample to inhibit angiotensinase and converting enzyme activity. The samples were generated for 2 hours at 39°C in a constant temperature water bath. All samples were assayed in triplicate and all samples from a given dog were assayed simultaneously. 125I angiotensin I (New England Nuclear) and antisera specific for angiotensin I (courtesy of Dr. Ted Goodfriend, University of Wisconsin) were added to each tube. Following 18 hours of equilibration at 4°C the bound and free angiotensin I was separated with dextran-coated
charcol. Free fractions and total counts were counted on an automated gamma counter (Nuclear Chicago) and expressed as percent free (free/total counts). A comparison of data from the plasma samples with a standard curve determined for each assay provided the quantity of angiotensin I that was produced. Nonspecific plasma binding was standardized by adding 50 microliters of angiotensin free plasma, obtained from nephrectomized dogs, to each point of the standard curve. This procedure assured that nonspecific plasma binding of $^{125}$I angiotensin was constant between points on the standard curve and plasma samples. Plasma renin activity is expressed as ng of angiotensin I formed per ml of plasma per hour of generation. With this procedure nonspecific plasma binding of $^{125}$I angiotensin was constant between points of the standard curve and plasma samples.

**EXPERIMENTAL PROTOCOL**

An identical protocol was followed in both the control dogs and those with prior beta adrenergic blockade. In the beta blocked group, propranolol (2 mg/Kg i.v.) was administered 15 minutes prior to the initiation of experimental procedures. In all dogs, a 30 minute equilibration period was observed prior to the initiation of any control of experimental procedures. During this period, a uniform initial blood gas state and a standardized relative minute ventilation between animals was established by setting ventilatory rate at 15 breaths per minute and adjusting tidal volume to achieve a 5% end expiratory $CO_2$ level. The minute ventilation thus determined was maintained throughout all control and experimental periods. Small doses of succinylcholine (Anectin) adequate for muscle relaxation were given intravenously as needed to suppress active ventilatory effort. Following the period of equilibration, a 30 minute initial control period was observed during which the dog was ventilated with room air. During the final two minutes of the period, initial control observations of blood pressure, heart rate and renal blood flow were made. Over the final minute a two ml arterial blood sample was drawn for plasma renin activity and hematocrit. An additional 2 ml sample was drawn into a heparinized syringe for arterial pH and blood gas determinations. In order to avoid error in blood gas analysis, air contamination was carefully avoided and determinations were done immediately after sampling. The dog was then ventilated for 30 minutes with 4% $CO_2$ in air at the minute ventilation previously described. An identical sequence of observations and blood samples was conducted as described for the control period. A similar 30 minute period of 8% $CO_2$ in air was followed by 12% $CO_2$ in air for another 30 minutes. After the completion of the 12% $CO_2$ study a recovery period was observed during which the dog was ventilated with room air until the end expiratory $CO_2$ had returned to the 5% control levels.

**RESULTS**

In the 8 dogs of the control group, mean plasma renin activity increased from an initial value of $1.59 \pm 0.75$ (SE) ng/ml/hr to $2.42 \pm 0.55$ ng/ml/hr following 30 minutes of 4% $CO_2$ inhalation, to $5.75 \pm 1.47$ ng/ml/hr following 30 minutes of 8% $CO_2$ inhalation and to $11.45 \pm 3.11$ ng/ml/hr following 30 minutes breathing of 12% $CO_2$. A recovery period of room air inhalation resulted in a decline in plasma renin activity to $4.34 \pm 2.12$ ng/ml/hr (Table I). In the 8 dogs of the propranolol group (Table II), mean plasma renin activity increased from an initial value of $1.79 \pm 0.69$ ng/ml/hr to $3.30 \pm 1.05$ ng/ml/hr following 30 minutes of 4% $CO_2$ inhalation, to $5.47 \pm 1.48$ ng/ml/hr following 30 minutes breathing of 8% $CO_2$ and to $7.96 \pm 2.94$ ng/ml/hr after 30 minutes of 12% $CO_2$ inhalation. A recovery period of room air inhalation resulted in a decline in plasma renin activity to $3.60 \pm 1.94$ ng/ml/hr. A time variant analysis applied to the control and propranolol groups across the treatments allowed a rejection of the null hypothesis that the renin treatment means were equal ($p < 0.001$). Figure 1 shows the mean response of all animals. A Tukey comparison of means indicated that plasma renin activity during 8% $CO_2$ and 12% $CO_2$ inhalation was significantly increased from control levels ($p < 0.001$). During 12% $CO_2$ inhalation the increase in plasma renin activity of the control dogs was significantly greater than that of the propranolol-treated dogs ($p < 0.05$). However, during 4% $CO_2$ and 8% $CO_2$ inhalation plasma renin activity levels were not different between control and propranolol groups.

Controlled inhalation of $CO_2$ produced the anticipated acidemia and elevations of $PaCO_2$. In the control dogs, inhalation of room air at minute ventilations set to produce end expiratory $CO_2$ of 5% resulted in mean $PaCO_2$ of 34.2 $\pm 1.03$ mmHg. In this group, $PaCO_2$ levels rose
TABLE I  RESPONSE OF CARDIOVASCULAR PARAMETERS AND ARTERIAL pH, PaCO₂ AND RENIN ACTIVITY DURING CONTROLLED CO₂ INHALATION IN CHLORALOSE ANESTHETIZED DOGS, (N = 8)

<table>
<thead>
<tr>
<th></th>
<th>0% CO₂</th>
<th>4% CO₂</th>
<th>8% CO₂</th>
<th>12% CO₂</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>130± 5.6</td>
<td>131± 5.3</td>
<td>112± 7.9</td>
<td>116± 6.2</td>
<td>132± 6.7</td>
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<tr>
<td>(mmHg)**</td>
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<tr>
<td>Heart rate</td>
<td>125±10.0</td>
<td>108± 6.3</td>
<td>81± 4.1</td>
<td>84± 5.3</td>
<td>132± 6.7</td>
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<tr>
<td>(B/min)**</td>
<td></td>
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<tr>
<td>Renal blood flow</td>
<td>161±20.0</td>
<td>173±17.0</td>
<td>162±20.2</td>
<td>137±22.0</td>
<td>187±21.9</td>
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<tr>
<td>(ml/min)*</td>
<td></td>
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<td></td>
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<tr>
<td>Renal vascular resistance</td>
<td>0.79±0.09</td>
<td>0.66±0.05</td>
<td>0.64±0.08</td>
<td>0.83±0.13</td>
<td>0.71±0.04</td>
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<tr>
<td>(mmHg/ml/min⁻¹)</td>
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<tr>
<td>Arterial pH***</td>
<td>7.37±0.01</td>
<td>7.27±0.08</td>
<td>7.13±0.02</td>
<td>7.03±0.01</td>
<td>7.36±0.01</td>
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<tr>
<td>Plasma renin activity</td>
<td>1.59±0.75</td>
<td>2.42±0.55</td>
<td>5.75±1.47</td>
<td>11.45±3.11</td>
<td>4.34±2.12</td>
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<tr>
<td>(ng/ml/hr⁻¹)**</td>
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</table>

A time variant analysis was applied across the experimental periods for each variable. The P values shown represent the level of confidence that a change had occurred during the course of the experimental period.
Mean ±(SE)  *p < 0.05,  **p < 0.01,  ***p < 0.005

TABLE II  RESPONSE OF CARDIOVASCULAR PARAMETERS AND ARTERIAL pH, PaCO₂ AND RENIN ACTIVITY DURING CONTROLLED CO₂ INHALATION IN CHLORALOSE ANESTHETIZED DOGS WITH PRIOR BETA ADRENERGIC BLOCKADE, (N = 8)

<table>
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<tr>
<th></th>
<th>0% CO₂</th>
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<th>8% CO₂</th>
<th>12% CO₂</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>124± 5.0</td>
<td>119± 4.7</td>
<td>107± 4.5</td>
<td>118± 5.6</td>
<td>138± 4.6</td>
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<tr>
<td>(mmHg)*</td>
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</tr>
<tr>
<td>Heart rate</td>
<td>113± 4.3</td>
<td>100± 6.6</td>
<td>98± 8.4</td>
<td>105±10.5</td>
<td>111± 8.1</td>
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<td>(B/min)</td>
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<tr>
<td>Renal blood flow</td>
<td>169±24.2</td>
<td>181±30.2</td>
<td>221±34.8</td>
<td>167±18.4</td>
<td>144±20.7</td>
</tr>
<tr>
<td>(ml/min)*</td>
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<tr>
<td>Renal vascular resistance</td>
<td>0.79±0.11</td>
<td>0.82±0.17</td>
<td>0.62±0.11</td>
<td>0.75±0.11</td>
<td>1.19±0.28</td>
</tr>
<tr>
<td>(mmHg/ml/min⁻¹)*</td>
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<tr>
<td>Arterial pH***</td>
<td>7.40±0.01</td>
<td>7.27±0.02</td>
<td>7.12±0.01</td>
<td>6.97±0.005</td>
<td>7.36±0.01</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)**</td>
<td>33.2±0.78</td>
<td>46.4±1.72</td>
<td>70.3±2.11</td>
<td>87.3±2.29</td>
<td>34.6±0.87</td>
</tr>
<tr>
<td>Plasma renin activity</td>
<td>1.79±0.69</td>
<td>3.30±1.05</td>
<td>5.47±1.48</td>
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A time variant analysis was applied across the experimental periods for each variable. The P values shown represent the level of confidence that a change had occurred during the course of the experimental period.
Mean ±(SE)  *p < 0.05,  **p < 0.01,  ***p < 0.005

To 45.2 ± 1.58 mmHg, 70.6 ± 2.88 mmHg and 89.8 ± 2.49 mmHg during inhalation of 4%, 8% and 12% CO₂ respectively. Room air recovery resulted in a return to approximately control values. Statistical analysis indicated that PaCO₂ following CO₂ inhalation is elevated from control values (p < 0.001). A Tukey comparison among means indicates that PaCO₂ during 8% and 12% CO₂ inhalation is significantly elevated from both control levels and levels achieved during 4% CO₂ inhalation. Mean arterial pH decreased from control values of 7.371 ± 0.01 to 7.125 ± 0.02 and to 7.032 ± 0.01 during 8% and 12% CO₂ inhalation. Blood gas and pH responses in the propranolol-treated dogs were similar to those of the control groups. As shown

DISCUSSION

It was reported earlier\(^1\) that plasma renin activity increases during 4% and 8% CO\(_2\) inhalation and it was suggested that the increased circulating renin had been a result of an altered state of blood gases and/or pH rather than renal hemodynamics. In the present study, during which dogs were also ventilated with 12% CO\(_2\), there was an added increment in circulating renin beyond that seen with 8% CO\(_2\). In fact, in the untreated dogs renin activity during 12% CO\(_2\) inhalation was double that seen with 8% CO\(_2\). The precise mechanisms are unclear but could conceivably be related to multiple factors known to elicit renin release. The release to renin from the kidney is thought to be modified by intra-renal baroreceptor and tubular mechanisms and by the sympathetic nervous system.\(^8\)

On the one hand Simmons\(^9\) and Minamisono et al\(^9\) concluded that the hemodynamic responses of the kidney to respiratory acidosis are attributable to neurohumoral factors originated by sympatho-adrenal stimuli. Animals with a similar fall in pH produced by CO\(_2\) retention presented evidence suggesting marked sympathtic-adrenal medullary stimulation, such as increased blood pressure, anuria, elevated catecholamine and blood glucose levels.\(^{14,15}\) Accordingly, it seems probable that in the present study the increased plasma renin activity may have been a result of sympatho-adrenal activation during acute respiratory acidosis. Morita\(^6\) however, has reported that the elevation of plasma renin activity during acute respiratory acidosis induced by CO\(_2\) inhalation is not blocked by hexamethonium administration. Thus it appears that the primary signal for renin activation under these conditions is post-ganglionic.

Propranolol has been reported to suppress renin secretion\(^8\) possibly by an effect on beta adrenergic receptors at sympathetic nervous terminals in the kidney.\(^16\) The renin response during CO\(_2\) inhalation suggests that the response may have been mediated by the action of increased circulating catecholamines acting on beta adrenergic receptors of the renin secretory process. The present studies of the CO\(_2\) response in propranolol-treated dogs was designed to test this idea. As shown in Figure 1, propranolol did not inhibit the increase of plasma renin during 4% and 8% CO\(_2\) inhalation, but during 12% CO\(_2\) inhalation a degree of suppression was evident. It appears, therefore, that during CO\(_2\) inhalation,

\[\text{Table II, PaCO}_2\text{ during 8% and 12% CO}_2\text{ inhalation is significantly elevated (p < 0.005) from both initial levels and levels achieved during 4\% CO}_2\text{ inhalation. Mean arterial pH decreased from initial values of 7.397 ± 0.01 to 7.121 ± 0.01 and to 6.971 ± 0.005 during 8\% and 12\% CO}_2\text{ inhalation.}\]

In the control and propranolol groups, mean arterial pressure, renal blood flow, heart rate and renal vascular resistance were not statistically altered during CO\(_2\) inhalation. Cardiovascular and blood gas and pH data are summarized in Table I and II. At no time in any of these studies was there evidence of hypoxemia. Blood PaO\(_2\) levels remained above 90 mmHg throughout.

In the propranolol groups, heart rate was not altered by administration of isoproterenol after the recovery.

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non-beta adrenergic mechanisms account for the major fraction of renin release. It is possible that in the non-treated dogs the added increment of renin during 12% CO₂ inhalation was a result of a somewhat greater reduction in arterial pressure, since it has been reported that even a modest fall in arterial blood pressure can increase renin secretion. Other evidence suggesting an intrarenal baroreceptor influence may be found from the calculated intrarenal vascular resistance. In both groups CO₂ inhalation of 4% and 8% was accompanied by reductions in renal vascular resistance, giving evidence that renal autoregulatory mechanisms were activated. There is good evidence that when renal autoregulatory influences are blocked by papaverine, intrarenal baroreceptor renin control is concomitantly blocked. The vasoactive properties of CO₂ and/or pH may have at least partially been responsible for the increased renin we observed. These factors may have influenced the intrarenal baroreceptor to release renin independent of beta adrenergic mechanisms. On the other hand, after 12% CO₂ inhalation renal vascular resistance in both groups increased to higher levels than that seen during the 4% and 8% CO₂ inhalation. This might be expected to have been the case following adrenal medullary activation. It appears likely that the major sympatho-adrenal activation had occurred during 12% CO₂ inhalation. This would also be consistent with the fact that propranolol had partially blocked the renin response at that time, since increased circulating catecholamines would be predicted to activate a beta adrenergic mediated release of renin by their direct action on juxtamedullary cells. On this basis we would predict that in the untreated dogs the release of renin during 12% CO₂ inhalation is a combination of intrarenal vasoactive influences and enhanced circulating catecholamines, the catecholamines playing a dual role via their vasoactive and direct juxtaglomerular influences. In the propranolol-treated group, direct juxtaglomerular beta adrenergic influences were blocked but vasoactive catecholamine influences remained.

Finally it should be reiterated that even though our studies showed that beta adrenergic influences are present, other non-sympathetic factors appear to play a major role in the release of renin during CO₂ inhalation. Furthermore, it appears that different renin control mechanisms are activated as PaCO₂ is progressively elevated.

REFERENCES