Mechanism of Suppression of Renin Secretion by Clonidine in the Dog

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SUMMARY The mechanism by which clonidine suppresses renin secretion was investigated in pentobarbital-anesthetized dogs in which renal perfusion pressure was controlled by means of an aortic clamp. Clonidine (30 μg/kg, iv) lowered mean arterial pressure (MAP) from 124 ± 8 to 104 ± 4 mm Hg (P < 0.01) and reduced plasma renin activity (PRA) to 32 ± 4% of the control value (P < 0.01) after 60 minutes. Ganglion blockade with pentolinium (3 mg/kg, iv) increased MAP from 148 ± 7 to 117 ± 3 mm Hg (P < 0.01) and reduced PRA to 55 ± 13% of the control value (P < 0.05) after 45 minutes. Pentolinium converted the hypotension produced by clonidine to hypertension (108 ± 9 to 146 ± 10 mm Hg at 60 minutes, P < 0.05) and abolished the suppression of PRA (105 ± 14% of control at 60 minutes, P > 0.05). In a further series of experiments, the effects of oxymetazoline, an α-adrenergic receptor agonist which is closely related to clonidine but which does not cross the blood brain barrier, were studied. Oxymetazoline (10 μg/kg, iv) increased MAP from 127 ± 3 to 154 ± 2 mm Hg (P < 0.01) and elevated PRA to 176 ± 22% of the control value (P < 0.02) after 30 minutes. A higher dose of oxymetazoline (30 μg/kg) increased MAP from 129 ± 10 to 161 ± 9 mm Hg (P < 0.05) and increased PRA to 256 ± 37% of control (P < 0.05) after 30 minutes. Taken together, these data support the hypothesis that the inhibition of renin secretion by clonidine results from a centrally mediated decrease in sympathetic neural activity.

SEVERAL INVESTIGATORS have reported that the hypotenison and bradycardia produced by clonidine are accompanied by a reduction in renin secretion.1-4 Considerable evidence suggests that the clonidine-induced decreases in blood pressure and heart rate are effected by a centrally mediated decrease in sympathetic tone,2 although peripheral actions to increase afferent baroreceptor activity4 may also contribute.

The suppression of renin secretion by clonidine also appears to result from decreased sympathetic activity. It is known that renal denervation decreases renin secretion4
and that clonidine reduces renal nerve activity. It has also been shown that renal denervation abolishes the suppression of renin secretion by clonidine. The sympato-inhibitory effect of clonidine is largely due to a central action of the drug, and there is evidence that the suppression of renin activity is also centrally mediated. For example, injection of clonidine into the cisterna magna or the 3rd ventricle of dogs, in doses which are ineffective when administered intravenously, decreases plasma renin activity.

On the other hand, recent evidence suggests that clonidine may also suppress renin release via a peripheral mechanism. For example, clonidine has been reported to inhibit renin secretion in rats after ganglion blockade or when infused into the isolated perfused rat kidney. However, clonidine does not inhibit renin release by rat kidney slices in vitro.

The aim of the present study was to evaluate further the hypothesis that the suppression of renin secretion by clonidine is due, at least in part, to an action of the drug in the central nervous system and that this action is mediated via a decrease in sympathetic neural tone. Two series of experiments were performed. In the first series, the effect of ganglionic blockade on the inhibition of renin secretion by clonidine was examined. If this inhibition is due to a decrease in sympathetic neural tone, then it would be expected that ganglionic blockade would also inhibit renin secretion and abolish the renin-lowering action of clonidine. In the second series of experiments, the effect of oxymetazoline, an α-adrenergic receptor agonist which is closely related to clonidine but which does not cross the blood-brain barrier, was tested. This compound would be expected to mimic the peripheral but not the central effects of clonidine. In all experiments, renal perfusion pressure was controlled to minimize changes in renin secretion resulting from changes in arterial pressure.

**Methods**

**Surgical Procedures**

Twenty-four mongrel dogs of either sex weighing 12-30 kg were studied. Prior to the experiments, the dogs were fed a diet which provided 70 mEq sodium per day. The dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv). A Blalock clamp was placed around the aorta above the origin of both renal arteries. By adjusting the clamp, it was possible to counteract the influence of changes in systemic arterial pressure and thus maintain a constant perfusion pressure below the clamp. A brachial and a femoral artery were cannulated to allow measurement of arterial pressure above and below the aortic clamp. Three experimental protocols were followed:

**Intravenous Clonidine**

The effect of intravenous clonidine (Boehringer Ingelheim Ltd.) in a dose of 30 μg/kg was studied in six dogs. Two blood samples separated by 15 minutes were collected prior to clonidine administration, and additional samples were collected 15, 30, and 60 minutes after the injection. Renal perfusion pressure, as reflected by femoral arterial pressure, was maintained at a constant level throughout the experiment by appropriate adjustment of the aortic clamp.

**Pentolinium and Clonidine**

Six dogs received intramuscular injections of the ganglionic blocking drug, pentolinium (Ansolysen; Wyeth), in two equal doses of 1.5 mg/kg administered 30 minutes apart. Blood samples were collected before and during the 45-minute period following the first injection. In three of these dogs and in another three similarly pretreated, clonidine (30 μg/kg, iv) was injected 45 minutes after the first dose of pentolinium, and blood samples were collected for the next 60 minutes, as described above. Renal perfusion pressure was held constant during this period. Sixty minutes after the clonidine injection, the aortic clamp was released so that the renal perfusion pressure increased to the systemic arterial level. A further blood sample was collected 15 minutes later.

**Intravenous Oxymetazoline**

The effect of intravenous oxymetazoline (Afrin; Schering Corp.) was studied in 12 dogs. Six of these received a dose of 10 μg/kg and six received a dose of 30 μg/kg. Blood samples were collected before and during the 60 minutes following oxymetazoline administration, as described above for intravenous clonidine. Renal perfusion pressure was held constant throughout the experiment.

Plasma renin activity was measured using a radioimmunoassay for angiotensin I and expressed as nanogram of angiotensin I formed per milliliter plasma during a 3-hour incubation (ng/ml per 3 hours).

All results are expressed as the mean ±SEM. The renin data are presented as a percentage of the control value. For this purpose, the control value was defined as the mean of the -15-minute and 0-minute control values. Statistical evaluation was performed using the paired t-test.

**Results**

**Intravenous Clonidine**

The effects of intravenous clonidine on blood pressure, heart rate, and plasma renin activity are summarized in Figure 1. Clonidine produced an initial rise in mean arterial pressure which lasted 20-30 minutes and was followed by a hypotensive response. Sixty minutes after the injection, mean brachial pressure had decreased from the control value of 124 ± 8 mm Hg to 104 ± 4 mm Hg (P < 0.01). Mean femoral pressure was maintained at 95 ± 2 mm Hg throughout the experiment. The changes in arterial pressure produced by clonidine were accompanied by decreases in heart rate from 131 ± 10 to 79 ± 5, 79 ± 6, and 86 ± 7 beats/min at 15, 30, and 60 minutes, respectively, after the clonidine injection (P < 0.01). Plasma renin activity was reduced to 61 ± 6%, 35 ± 4%, and 32 ± 4% of the control value (22.1 ± 7.8 ng/ml per 3 hours) 15, 30, and 60 minutes, respectively, after clonidine (P < 0.05).

**Pentolinium and Clonidine**

The effects of ganglionic blockade with intramuscular pentolinium are summarized in Figure 2. Pentolinium
FIGURE 1  The effect of clonidine on mean arterial pressure, heart rate, and plasma renin activity in pentobarbital-anesthetized dogs (n = 6). Control plasma renin activity = 22.1 ± 7.8 ng/ml per 3 hours.

FIGURE 2  The effect of ganglion blockade with pentolinium on mean arterial pressure, heart rate, and plasma renin activity in pentobarbital-anesthetized dogs (n = 6). Control plasma renin activity = 29.8 ± 9.8 ng/ml per 3 hours.

FIGURE 3  The effect of clonidine on mean arterial pressure, heart rate and plasma renin activity following ganglion blockade with pentolinium in pentobarbital-anesthetized dogs (n = 6). Control plasma renin activity = 23.5 ± 8.2 ng/ml per 3 hours.

produced a fall in mean brachial pressure from 148 ± 7 to 117 ± 3 mm Hg (P < 0.01) and in heart rate from 131 ± 12 to 117 ± 11 beats/min (P < 0.01) 45 minutes after the first injection. During this period, mean femoral pressure was maintained at 90 ± 2 mm Hg. The pentolinium-induced cardiovascular changes were accompanied by a reduction in plasma renin activity to 72 ± 8%, 61 ± 13%, and 55 ± 13% of the control value (29.8 ± 9.8 ng/ml per 3 hours) 15, 30, and 45 minutes, respectively, after the injection (P < 0.05).

Pentolinium markedly altered the cardiovascular and renin secretory responses to intravenous clonidine (Fig. 3). The magnitude and duration of the clonidine-induced pressor response were increased after pentolinium so that 60 minutes after the clonidine injection mean arterial pressure, 146 ± 10 mm Hg, was still significantly greater than the control value (108 ± 9 mm Hg) (P < 0.05). In addition, the decrease in heart rate produced by clonidine was virtually abolished by pentolinium.

As well as altering the cardiovascular responses to clonidine, pentolinium abolished the clonidine-induced suppression of plasma renin activity. In each of the pentolinium-treated dogs, plasma renin activity increased after clonidine. The magnitude of this response ranged between 132% and 891% of the control value (23.5 ± 8.2 ng/ml per 3 hours). The mean increase was to 302 ± 125%, 195 ± 50%, and 105 ± 14% of the control at 15, 30, and 60 minutes, respectively, but because of the...
variability of the response, these changes were not statistically significant.

Following release of the aortic clamp 60 minutes after clonidine, mean femoral arterial pressure increased from 83 ± 2 to 124 ± 11 mm Hg (P < 0.01) and plasma renin activity decreased from 105 ± 14% to 59 ± 11% of control (P < 0.03) (Fig. 4).

**Intravenous Oxymetazoline**

Oxymetazoline (10 µg/kg, iv) increased mean brachial pressure from 127 ± 3 to 164 ± 3 mm Hg after 15 minutes (P < 0.01) and to 154 ± 2 mm Hg after 30 minutes (P < 0.01) (Fig. 5). By 60 minutes, arterial pressure had decreased to a value not significantly different from the control value. Mean femoral arterial pressure was maintained at 92 ± 2 mm Hg throughout the experiment. There was no significant change in heart rate. Plasma renin activity increased to 210 ± 30% and 176 ± 22% of the control value (14.4 ± 3.8 ng/ml per 3 hours) 15 and 30 minutes, respectively, after oxymetazoline (P < 0.02). At 60 minutes, plasma renin activity had decreased to a value that was not significantly different from control (P > 0.2).

A higher dose of oxymetazoline (30 µg/kg, iv) produced changes similar to those produced by the lower dose (Fig. 6). Mean brachial pressure rose from 129 ± 10 to 172 ± 8 mm Hg after 15 minutes (P < 0.05) and to 161 ± 9 mm Hg after 30 minutes (P < 0.05). At 60 minutes, the brachial pressure was not significantly different from the control value. Heart rate did not change significantly during this period. Plasma renin activity increased to 258 ± 57%, 256 ± 37%, and 216 ± 27% of the control value (27.1 ± 6.7 ng/ml per 3 hours) 15, 30, and 60 minutes, respectively, after oxymetazoline (P < 0.05).
Discussion

It is now well established that the hypotension and bradycardia produced in man and experimental animals by clonidine is accompanied by suppression of renin secretion. The cardiovascular effects of clonidine appear to result from an action of the drug within the central nervous system to decrease sympathetic neural activity. Previous studies in this and other laboratories suggest that a similar mechanism is responsible for the suppression of renin secretion, i.e., that the suppression results from a centrally mediated decrease in renal sympathetic neural tone. The evidence on which this hypothesis is based may be summarized as follows: (1) Central administration of clonidine, in doses which are ineffective when injected intravenously, produces significant suppression of renin secretion. (2) Clonidine reduces spontaneous renal sympathetic nerve activity. (3) The suppression of renin secretion by intravenous clonidine is prevented by renal denervation.

The results of the present study provide additional support for the concept that the suppression of renin secretion by clonidine is due to its central sympatholytic action. Administration of the ganglionic blocking drug, pentolinium, produced a reduction of plasma renin activity similar to that produced by clonidine. The ability of pentolinium to decrease plasma renin activity is consistent with previous reports that ganglionic blockade inhibits the increase in renin secretion produced by hemorrhage, hypotension, and exercise. Further and demonstrates the important role of the sympathetic nervous system in the control of renin secretion. In addition to decreasing plasma renin activity, pentolinium abolished the inhibition of renin secretion by clonidine. It might be argued that the failure of clonidine to inhibit renin secretion after pentolinium was due to the fact that pentolinium had already produced maximum suppression of renin secretion. This explanation seems unlikely, however, since further falls in plasma renin activity were readily produced in the ganglion-blocked dogs when renal perfusion pressure was increased (Fig. 4). Taken together, these results provide additional support for the hypothesis that the clonidine-induced suppression of renin secretion results from the sympathetic-inhibitory action of the drug.

The present results differ from those of Pettinger et al., who were unable to block the renin-lowering action of clonidine in rats by ganglionic blockade. This discrepancy is probably due, at least in part, to the fact that in their experiments, drug-induced changes in arterial pressure were transmitted to the kidney, whereas, in the present study, changes in renal perfusion pressure were minimized by means of an aortic clamp. This is an important difference, since it is well established that there is an inverse relationship between renal perfusion pressure and the rate of renin secretion. This is illustrated in the experiments of Pettinger et al. who observed that ganglionic blockade lowered arterial pressure and stimulated renin secretion, whereas, in our experiments in which renal perfusion pressure was held constant, ganglionic blockade produced the expected fall in plasma renin activity. The pressor effect of clonidine is potentiated by ganglionic blockade, and this may have contributed substantially to the fall in plasma renin activity produced by clonidine in ganglion-blocked rats. This proposal is supported by the finding of Pettinger et al. that a-adrenergic blockade with phenolamine, which is known to antagonize the pressor action of clonidine, prevented the clonidine-induced suppression of renin secretion in their ganglion-blocked animals.

The finding in the present study that the clonidine-induced decrease of plasma renin activity is abolished by ganglionic blockade supports the hypothesis that the suppression results from decreased sympathetic activity. However, these results do not indicate whether the decrease in sympathetic activity results from a central or a peripheral action of the drug. To distinguish between these two possibilities, the effect on renin secretion of intravenous oxymetazoline was studied. This imidazoline derivative is closely related to clonidine but, due to its low lipid solubility, does not cross the blood-brain barrier. It would therefore be expected that oxymetazoline would mimic the peripheral but not the central effects of clonidine. Administered intravenously in a dose of 30 μg/kg, oxymetazoline produced a prolonged pressor response and an increase in plasma renin activity. Administration of a lower dose, 10 μg/kg, which on the basis of published data would be expected to be equi-effective as an a-adrenergic agonist as 30 μg/kg clonidine, also increased arterial pressure and plasma renin activity. The data thus support the hypothesis that the suppression of renin secretion by clonidine results from an action of the drug within the central nervous system.

The mechanism responsible for the increase in plasma renin activity observed following both doses of oxymetazoline and following clonidine in ganglion-blocked dogs was not established in the present study. However, a likely possibility is that the increase was due to renal vasoconstriction which could stimulate renin secretion via the renal baroreceptor or macula densa mechanisms. Therefore, clonidine may have two effects on renin secretion, i.e., an inhibitory effect which results from decreased renal sympathetic nerve activity and a stimulatory effect which is due to renal vasoconstriction. The former component appears to predominate under normal conditions so that the net effect of clonidine is inhibition of renin secretion. On the other hand, oxymetazoline only produces vasoconstriction and stimulation of renin secretion.

The results of the present study do not exclude the possibility that clonidine might inhibit renin secretion via a direct intrarenal action as suggested by Pettinger et al. and Vandongen and Greenwood. However, the results do indicate that, in the pentobarbital-anesthetized dog, the inhibition of renin secretion by clonidine results primarily from a centrally mediated decrease in renal sympathetic nerve activity.

It should be noted that the dose of clonidine used in the present study is considerably larger than the doses used clinically. Furthermore, under the experimental conditions of pentobarbital anesthesia, characterized by a high level of sympathetic neural tone and a reduced renal perfusion pressure, the basal level of plasma renin activity is higher than in unanesthetized animals. In view of these considerations, extrapolation of the present data to clinical circumstances should be made with caution.
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References

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