Effects of Clonidine on Central and Peripheral Nerve Tone in Primary Hypertension

PATRICK A. SULLIVAN, VINCENT DE QUATTRO, ANDRAS FOTI, AND GERALD CURZON

SUMMARY To define the mechanisms whereby clonidine lowers blood pressure, we measured cerebrospinal fluid and plasma levels of norepinephrine, normetanephrine, epinephrine, dopamine, and the dopamine metabolite homovanillic acid in 10 primary hypertensive subjects before and after 3 months of clonidine treatment (mean dose, 0.68 mg/day). Catecholamines were measured by radioenzymatic methods. Cerebrospinal fluid and plasma sampling was performed after subjects had fasted and remained supine overnight, and plasma sampling was repeated 2 hours later, after subjects had ambulated. Supine and upright blood pressure fell, as might be expected. Cerebrospinal fluid levels of norepinephrine and normetanephrine fell significantly, but dopamine and homovanillic acid levels were unchanged. Plasma norepinephrine, normetanephrine, and epinephrine levels decreased 30 to 50%, and supine dopamine levels also fell. The percent fall in supine blood pressure was related to the fall of cerebrospinal fluid and plasma norepinephrine. There were also positive relationships between the decreases of plasma norepinephrine and of normetanephrine and dopamine. The cerebrospinal fluid/plasma norepinephrine ratio was unaffected by clonidine, suggesting that the drug lowered both pools equally. Our findings indicate that clonidine decreases both central and peripheral norepinephrine activity. The dopaminergic activity of cerebrospinal fluid was unaffected by clonidine, and though plasma dopamine levels tended to be lower after treatment, mean plasma prolactin level, an index of dopaminergic activity, was also unchanged. The fall in plasma epinephrine level is probably related to diminished sympathetic adrenomedulillary stimulation and is unlikely to contribute to clonidine's antihypertensive action. These results also suggest that measurement of normetanephrine in cerebrospinal fluid and plasma provides a good index of norepinephrine activity.

(Hypertension 8: 611-617, 1986)

KEY WORDS • plasma catecholamines • cerebrospinal fluid • essential hypertension • effects of clonidine

THERE is evidence, albeit controversial, 1 that sympathetic nervous hyperactivity associated with raised circulating plasma catecholamine levels is involved in the pathogenesis of essential hypertension. 2-4 Norepinephrine (NE) is the principal neurotransmitter of the sympathetic nervous system 5 and is formed locally in the nerve terminals from the amino acid tyrosine and dopamine. Clonidine is reported to lower blood pressure through central mechanisms, 6,7 probably by stimulation of either central presynaptic α-adrenergic receptors 8,9 or central postsynaptic mechanisms. 10-13 Despite its presumably dominant central hypotensive action, most human studies with clonidine relate to its effects on peripheral catecholamine levels. 14-16 Martin et al., 17 however, reported that clonidine decreased NE levels and increased levels of the dopamine metabolite homovanillic acid (HVA) in the cerebrospinal fluid (CSF) of five normotensive subjects with amnesic disorders. Cubeddu et al. 18 recently found that clonidine lowered levels of NE, but not epinephrine (E) or dopamine (DA), in the CSF and plasma of hypertensive subjects, while Bertilsson et al. 19 described a clonidine-induced fall in levels of the NE metabolite 4-hydroxy-3-methoxyphenylglycol, suggesting a decreased release or turnover of NE in CSF.

Because catecholamines in plasma penetrate the blood-brain barrier poorly, 20-22 and brain areas rich in NE lie in close proximity to the CSF, 23-24 an estimation of the catecholamine concentrations in CSF should provide a reasonable index of central sympathetic nervous activity. We have previously shown that measurement of NE and normetanephrine (NMN) may
help to characterize sympathetic nerve tone in patients with primary hypertension. In this study, therefore, the main objective was to evaluate central neurochemical mechanisms by which clonidine may lower blood pressure in essential hypertension. In addition to studying its effects on central and peripheral noradrenergic activity, we also investigated whether clonidine modifies CSF concentrations of the NE precursor DA, the NE metabolite NMN, and the DA metabolite HVA. The effects of clonidine on blood pressure, plasma renin activity (PRA), and levels of plasma catecholamines, plasma prolactin (an index of dopaminergic activity), and plasma aldosterone were also investigated.

**Subjects and Methods**

Ten nonobese white subjects (6 men, 4 women) with a mean age of 58.1 (range, 34-71) years and a mean weight of 72.4 (range, 59.9-94) kg were studied at one center (Mallow General Hospital). Essential hypertension was diagnosed in all subjects on the basis of repeated outpatient blood pressure measurements in excess of 160/95 mm Hg. Pretreatment investigations included serum chemistry values, urinalysis, chest roentgenography, electrocardiography, rapid sequence intravenous urography, and 24-hour urinary catecholamine estimations. No subject had a history of congestive cardiac failure, myocardial infarction, cerebrovascular accident, diabetes mellitus, or renal disease. Two subjects had a history of excessive alcoholic consumption, but neither had consumed alcohol for at least 3 months before the study. Eight subjects had never received antihypertensive therapy, and the remaining two had all medications discontinued at least 2 weeks before the study was undertaken.

All subjects were hospitalized 48 hours before CSF sampling and were on an unrestricted ward diet. To eliminate the confounding influence of circadian variation of brain or spinal fluid NE,28 all subjects were studied between 0900 and 1030 after having fasted, abstained from smoking, and remained supine overnight. The mean of three measurements of supine blood pressure was recorded before sampling. A 30-ml sample of blood was drawn from an antecubital vein, and immediately thereafter CSF sampling was performed by one investigator (P.A.S.) with subjects lying in the lateral position. To minimize differences due to concentration gradients of NE,27 the first 6 ml of CSF was discarded and the subsequent 4 ml was obtained for study purposes. Blood and CSF were collected into iced tubes, and the former was immediately centrifuged. Both were subsequently frozen in liquid nitrogen. Because recent evidence suggests a lack of benefit from bed rest on the occurrence of post-lumbar puncture headache,28,29 blood pressure measurements and plasma sampling were repeated after 2 hours of ambulation with the subjects in the erect position. Clonidine treatment then was begun; the initial dosage was 0.1 mg t.i.d. and was increased 0.1 mg t.i.d. every 4 weeks until an outpatient blood pressure of 140/90 mm Hg or less or a dosage of 0.3 mg t.i.d. was achieved. The mean daily dose of clonidine used was 0.68 ± 0.3 (range, 0.3-0.9) mg. Subjects were re-studied after 3 months of treatment, between 2 and 3 hours after the morning dose of clonidine. There were three incidences of post-lumbar puncture headache. No other complications were associated with the procedures. Three subjects reported dry mouth, and one experienced depression and drowsiness during treatment.

The protocol was approved by the Mallow Hospital Ethics Committee. Informed consent for the procedures was obtained from all subjects after a full description of the protocol.

Preclonidine and postclonidine CSF and plasma samples were assayed simultaneously. Contents of NE and E in plasma and CSF were measured by the radioenzymatic method of Peuler and Johnson.30 Coefficients of variation for NE and E were 7.4% and 8.5%, respectively. The DA concentration was measured by a modification of the aforementioned method; the coefficient of variation was 13%. The NE metabolite NMN was measured according to the method of Kobayashi et al.31 The dopamine metabolite HVA was measured in CSF as described by Bridges et al.32 The PRA was measured by the method of Craven and Symonds33 and aldosterone was measured by a radioimmunoassay kit method (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma prolactin was measured as described by Lannigan and Powell.34

Mean values were compared using Student's paired t test. Relationships between variables were analyzed using Pearson correlation coefficients. Data were expressed as means ± SEM.

**Results**

Individual and mean blood pressures, catecholamine levels, and other variables and changes following treatment with clonidine are shown in Table 1 and Figures 1 and 2. Systolic and diastolic (supine and erect) blood pressures all fell significantly, although individual changes tended to vary greatly. Mean blood pressure decreases varied between 10 and 16%.

Levels of CSF NE and NMN showed modest but significant decreases with clonidine treatment. Individual values fell in all but one subject (Subject 8) who also had a limited blood pressure response to the drug. Mean CSF NE and NMN decreases were 30% and 41%, respectively, while CSF levels of DA and HVA did not change appreciably. Both supine and erect plasma NE levels fell significantly; mean decreases were virtually identical (36.2% and 36.6%, respectively) and were similar to the changes that occurred in CSF (30%). Plasma NMN decreases of 43.5% (supine) and 34.4% (erect) were also similar to those seen in CSF (41%). Plasma catecholamine values in Subject 8 were unchanged or slightly increased with treatment; these findings parallel those found in CSF.

Changes in plasma E levels after clonidine treatment paralleled those seen in NE and NMN, although the mean decrease appeared more marked at 51.3% (supine) and 48.9% (erect). Plasma DA values also tend-
ed to fall, but the mean value was significant only in the supine position. Plasma prolactin levels did not change appreciably (Table 2). Clonidine treatment significantly reduced upright PRA but otherwise had no significant effect on PRA or aldosterone levels (see Table 2).

There were no significant relationships between pretreatment blood pressures and either CSF or plasma catecholamine levels. After treatment, however, erect systolic blood pressure correlated with erect plasma NE ($r = 0.65$, $p < 0.05$) and E ($r = 0.68$, $p < 0.05$) levels. Supine diastolic blood pressure also correlated with supine plasma DA levels ($r = 0.71$, $p < 0.01$). The percentage decrease in supine systolic blood pressure correlated with the reduction in levels of both CSF NE ($r = 0.72$, $p < 0.01$) and supine plasma NE ($r = 0.60$, $p < 0.05$).

Levels of NE and NMN were positively related in CSF ($r = 0.62$, $p < 0.05$), supine plasma ($r = 0.53$, $p < 0.05$), and erect plasma ($r = 0.64$, $p < 0.01$). The percentage decrease in CSF NE levels correlated with that in supine plasma NE ($r = 0.64$, $p < 0.05$). No relationship was noted between the percentage change in NE and NMN levels in CSF, but the changes were significant in plasma samples (supine, $p < 0.05$; erect, $p < 0.01$). Highly significant relationships also were seen between the decrease in plasma levels of NE and DA (supine, $r = 0.81$, $p < 0.01$; standing, $r = 0.77$, $p < 0.01$) and of NE and E (supine, $r = 0.77$, $p < 0.01$). The change in CSF DA levels did not correlate with that of its metabolite HVA ($r = 0.34$).

**Discussion**

Our results confirm that clonidine monotherapy is effective in mild to moderate hypertension. Our findings are broadly similar to those reported by Martin et al., Cubeddu et al., and Bertilsson et al. However, one of these studies involved normotensive amnesic subjects, while another measured only catecholamine metabolites.

We attempted to minimize circadian and postural variations of brain and spinal fluid catecholamines by ensuring that all subjects were studied after prolonged bed rest and at the same time of the day. However, true basal catecholamine levels probably were not achieved due to the nature of the study. Despite this, both plasma and CSF NE levels decreased, suggesting that clonidine lowers both peripheral and central sympathetic nervous activity. Available evidence indicates that clonidine's blood pressure-lowering effect is predominantly central in origin and probably related to stimula-
TABLE 1. Effects of Clonidine on Individual Blood Pressure and Catecholamine Values and Group Differences

<table>
<thead>
<tr>
<th>Subject no., treatment</th>
<th>Blood pressure (mm Hg)</th>
<th>CSF (ng/L)</th>
<th>CSF HVA (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Erect</td>
<td>NE</td>
</tr>
<tr>
<td>1 Placebo</td>
<td>139/86</td>
<td>147/95</td>
<td>244</td>
</tr>
<tr>
<td>Clonidine</td>
<td>131/71</td>
<td>108/71</td>
<td>195</td>
</tr>
<tr>
<td>2 Placebo</td>
<td>150/92</td>
<td>142/96</td>
<td>235</td>
</tr>
<tr>
<td>Clonidine</td>
<td>131/71</td>
<td>142/89</td>
<td>151</td>
</tr>
<tr>
<td>3 Placebo</td>
<td>158/99</td>
<td>182/94</td>
<td>601</td>
</tr>
<tr>
<td>Clonidine</td>
<td>141/86</td>
<td>161/93</td>
<td>385</td>
</tr>
<tr>
<td>4 Placebo</td>
<td>197/117</td>
<td>211/109</td>
<td>430</td>
</tr>
<tr>
<td>Clonidine</td>
<td>181/95</td>
<td>167/97</td>
<td>278</td>
</tr>
<tr>
<td>5 Placebo</td>
<td>150/87</td>
<td>154/78</td>
<td>397</td>
</tr>
<tr>
<td>Clonidine</td>
<td>141/91</td>
<td>146/87</td>
<td>247</td>
</tr>
<tr>
<td>6 Placebo</td>
<td>196/103</td>
<td>140/93</td>
<td>317</td>
</tr>
<tr>
<td>Clonidine</td>
<td>168/88</td>
<td>143/88</td>
<td>268</td>
</tr>
<tr>
<td>7 Placebo</td>
<td>211/97</td>
<td>195/102</td>
<td>316</td>
</tr>
<tr>
<td>Clonidine</td>
<td>161/95</td>
<td>155/76</td>
<td>129</td>
</tr>
<tr>
<td>8 Placebo</td>
<td>158/105</td>
<td>180/105</td>
<td>315</td>
</tr>
<tr>
<td>Clonidine</td>
<td>155/101</td>
<td>155/106</td>
<td>396</td>
</tr>
<tr>
<td>9 Placebo</td>
<td>165/105</td>
<td>166/94</td>
<td>354</td>
</tr>
<tr>
<td>Clonidine</td>
<td>159/79</td>
<td>109/72</td>
<td>274</td>
</tr>
<tr>
<td>10 Placebo</td>
<td>180/92</td>
<td>190/97</td>
<td>388</td>
</tr>
<tr>
<td>Clonidine</td>
<td>142/80</td>
<td>123/65</td>
<td>188</td>
</tr>
<tr>
<td>Means ± SEM</td>
<td>170 ± 8/</td>
<td>171 ± 8/</td>
<td>360 ± 33</td>
</tr>
<tr>
<td></td>
<td>98 ± 3</td>
<td>96 ± 3</td>
<td>360 ± 33</td>
</tr>
<tr>
<td></td>
<td>151 ± 5*</td>
<td>141 ± 7*</td>
<td>251 ± 28*</td>
</tr>
<tr>
<td></td>
<td>86 ± 3*</td>
<td>84 ± 4*</td>
<td></td>
</tr>
</tbody>
</table>

CSF = cerebrospinal fluid; NE = norepinephrine; NMN = normetanephrine; DA = dopamine; HVA = homovanillic acid; E = epinephrine.

*p < 0.005, †p < 0.01, ‡p < 0.05, compared with placebo values.

ulation of α-adrenergic receptors, which results in decreased sympathetic outflow.8, 36-38

Could the changes in CSF catecholamine levels reflect what is happening to plasma catecholamines rather than indicate a central effect of clonidine on sympathetic nervous activity? The strikingly similar decreases in CSF and plasma NE (30% and 36%, respectively) and NMN (41% and 43%, respectively) and the associated significant correlations could be taken to reflect a passive exchange of catecholamines from plasma across the blood-brain barrier. Additionally, blood vessels in the pia arachnoid area and the choroid plexus are supplied by sympathetic nerves, so it is possible that CSF catecholamines arise from these nerves rather than from the brain.39 However, clonidine has been shown to reduce the rate of firing of NE cells in the locus ceruleus as well as brain NE turnover, as measured by 4-hydroxy-3-methoxyphenylglycol concentrations and the α-methyltyrosine-induced fall in NE content.40 Additionally, intravenously injected NE does not cross the blood-brain barrier22, 23, 41 and systemically injected DA and its metabolite HVA does not increase CSF HVA levels,44 but stimulation of dopaminergic neurons evokes release of
HVA into CSF. More recently, Wood found evidence that most of the HVA in CSF originates from brain parenchyma adjacent to the lateral ventricle. In a study on patients with pheochromocytoma, we also noted a barrier to the passage of NE from plasma to CSF.

It would seem reasonable, therefore, to assume that our CSF findings indicate a lowering of central sympathetic nervous activity and that the similar decreases in CSF and plasma catecholamine levels probably indicate an equal but separate effect of clonidine on both pools. The CSF to plasma NE ratios were also unchanged at 0.72 before and 0.79 after clonidine treatment. These ratios are broadly similar to those reported by Ziegler et al.

It is readily appreciated that the number of subjects studied was small and some of the changes noted could be due to a placebo effect. Indeed, a placebo control group of subjects studied under similar conditions would have been ideal; however, this would not have been possible given the circumstances of the current study. Previous placebo-controlled studies have shown that the antihypertensive effect of clonidine is similar to that found in our study. In the placebo-
controlled study of Martin et al.,17 the average reduction in levels of CSF and plasma NE was 70%.
Mean CSF DA and HVA values were unaffected by clonidine treatment, a finding similar to that reported by Bertilsson et al.19 This finding suggests that central dopaminergic activity is not influenced by the drug, and the similar plasma prolactin values before and after treatment also concur with this conclusion. In contrast, however, Martin et al.17 found increased CSF HVA concentrations after clonidine treatment and suggested that some of its antihypertensive action was related to this change.
Clonidine treatment decreased levels of NE and its metabolite NMN in CSF and plasma, and its antihypertensive effect appears to be mediated by a reduction in central sympathetic nerve outflow. Whether these changes reflect presynaptic α2-adrenergic or postsynaptic mechanisms cannot be determined from this study. Levels of NE and NMN were positively correlated not only in CSF but also in plasma, a result that we previously reported in hypertensive and control subjects.31 Our findings indicate that measurement of NMN in both plasma and CSF reflects changes in neuronal activity.

Acknowledgments
We thank Marian O’Brien for typing this manuscript and Dr. David Powell, Mater Hospital, Dublin, for measuring plasma prolactin levels.

References
3. De Quattro V, Chan S. Raised plasma catecholamines in some patients with primary hypertension. Lancet 1972;1:806-807
27. Carbbat PAT, van Creveld H. Lumbar puncture headache: con-
trol study on the preventative effect of 24 hours bed rest. Lancet 1981;2:1133—1135


40. Svensson TH, Bunney BS, Aghajanian GK. Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha adrenergic agonist, clonidine. Brain Res 1975;92:291—306


42. Rochette L, Bralet AM, Bralet J. Effects of chronic clonidine treatment on the turnover of noradrenaline and dopamine in various regions of the rat brain. Naunyn Schmiedebergs Arch Pharmacol 1982;319:40—42

43. Van Voert MH, Sethy VH. Therapy of intention myoclonus with L-5 hydroxytryptophan and a peripheral decarboxylase inhibitor, MK-486. Neurology (Minneapolis) 1975;25:135—140


45. Portig PJ, Vogt M. Release to the cerebral ventricles of substances with possible transmitter function into the caudate nucleus. J Physiol (Lond) 1969;204:687—715


50. Toubes DB, McIntosh T1, Kirkendall WM, Wilson WR. Hypotensive effects of clonidine and chlorthalidone controlled clinical trial on drugs administered singly and in combination. Am Heart J 1971;82:312—318

Effects of clonidine on central and peripheral nerve tone in primary hypertension.
P A Sullivan, V De Quattro, A Foti and G Curzon

Hypertension. 1986;8:611-617
doi: 10.1161/01.HYP.8.7.611

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/8/7/611

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/