Cryoblockade of the Ventromedial Frontal Cortex Reverses Hypertension in the Rat

JULIANNA E. SZILAGYI, ADDISON A. TAYLOR, AND JAMES E. SKINNER

SUMMARY The anteroventral part of the hypothalamus adjacent to the third ventricle (AV3V) has been implicated in electrolytic lesion studies as a site crucial to the development and maintenance of hypertension. Cryoblockade is known to alter synaptic and axonal transmission differently at different temperatures. In this study, cooling of the hypothalamus, including the AV3V area, to the temperature known to block only synaptic function did not alter blood pressure in two different models of experimental hypertension in the rat. Cooling sufficient to block both synaptic and axonal transmission, however, reduced blood pressure elevations to near normotensive levels. Synaptic cryoblockade in the ventromedial portion of the frontal cortex lowered experimental hypertension by 21 ± 3 mm Hg (p<0.05). In normotensive controls, blood pressure was not altered by cryoblockade in either the frontal cortex or hypothalamus. Anatomical evidence provided by others shows that cells in the ventromedial frontal cortex project, in part, through the AV3V region to the brainstem cardio-regulatory structures. These results indicate that neural activity arising in frontal cortex is axonally projected through the hypothalamus to maintain elevated blood pressure in experimental hypertension. (Hypertension 9: 576-581, 1987)

KEY WORDS • blood pressure • brain • anteroventral third ventricle • deoxycorticosterone acetate–salt • blood pressure

CENTRAL nervous system involvement in the development and maintenance of experimentally induced hypertension has been clearly demonstrated. For example, electrolytic lesions in the anteroventral part of the hypothalamus adjacent to the third ventricle (AV3V) prevent blood pressure elevations in renovascular and mineralocorticoid models of hypertension.1 Although such hypothalamic lesions do not alter the maintenance or development of high blood pressure in spontaneously hypertensive rats (SHR), bilateral lesions in the central amygdaloid nuclei attenuate the development of hypertension in these rats.2

Placement of a medullary lesion overlapping the nucleus of the tractus solitarius causes blood pressure elevations that can be reversed by cerebral transections above the level of the brainstem lesion.3 Thus, a concept has emerged in which higher cerebral centers above the mesencephalon are thought to project neural information to the brainstem vasomotor centers to produce or maintain elevated blood pressure.

An electrolytic lesion, such as that used to study the AV3V, blocks both the local synaptic activity and the propagation of action potentials between distant structures that are interconnected by axons passing through the damaged region. Both the frontal cortex and amygdala have descending fibers that run throughout the medial and lateral portions of the hypothalamus, including what appears to be the AV3V region.4,5 It is therefore unclear whether the antihypertensive effect produced by AV3V lesions is due to local damage or to destruction of the fibers of passage.

The use of cryoblockade enables a distinction to be made between these two possibilities. Brooks7 carefully documented the conclusion that cooling brain tissue in the range of 20 to 10°C will block synaptic transmission without altering axonal propagation, whereas cooling to the 10 to 0°C range will block both. The
cooling of cryoprobe surfaces to produce intracerebral
temperature gradients in each of these two ranges re-
results in locally circumscribed blockade effects that are
completely reversed following cessation of cooling and
rewarming of the tissue. These features of cryo-
blockade enable each subject to be observed during
conditions of control, synaptic blockade, and fiber
plus synaptic blockade, so that inferences can be
made.

We used this cryoblockade technique to provide evi-
dence to support the conclusion that the hypothalamic
lesion effects mentioned above resulted from blockade
of fibers of passage. Since some of the blocked hypo-
thalamic axons appear to arise from neurons in the
ventromedial prefrontal cortex, we investigated the
possibility that blockade at this cortical locus also re-
duces blood pressure elevations. Cryoblockade effects
were observed in two different models of experimental
hypertension in the rat.

Materials and Methods

A total of 28 Sprague-Dawley rats (200–250 g; Har-
lan Industries, Houston, TX, USA) were used in the
study. Institutional guidelines were followed for all
procedures. Animals randomly selected for mineralo-
corticoid hypertension were anesthetized with meth-
oxyflurane, and after unilateral nephrectomy, a Silas-
100 mg/kg, or an isotonic vehicle placebo
plantation, each control and hypertensive rat was again

Renovascular hypertension was induced by aortic
cocarctation. The anesthetized rats were unilaterally
nenephrectomized, and the abdominal aorta was con-
stricted just above the renal artery of the remaining
kidney. The aorta was ligated with a 22-gauge hypo-
dermic needle (2-mm diameter); the needle was then
removed to allow limited aortic flow. The develop-
ment of the hypertension was monitored by the tail cuff
method.

One week before the experiment, all control and
hypertensive rats were reanesthetized and implanted
with one or more cryoprobes, 1 mm in diameter, of the
type previously used in our laboratory. One midline
cryoprobe was stereotaxically placed in targets at 1)
the bottom of the third ventricle, on the midline, rang-
ing from the posterior to the anterior hypothalamus,
including the AV3V region; 2) the thalamus dorsal to
the hypothalamic placements; and 3) the dorsomedial
cortex, just anterior to the genu of the corpus callosum.

Bilateral (two) cryoprobes were positioned in 1) the
ventromedial cortex, just anterior to the genu of the
corpus callosum, and 2) the frontal pole, several milli-

The thalamic and dorsomedial frontal cortex place-
ments were selected as control sites. That is, these
control placements had intracerebral tracks that were
congruent with the shaft portion of the tracks of the
experimental placements whose tips were targeted at
the hypothalamus and ventromedial frontal cortex.

This matched-pair technique enables determination
that the reduction of elevated blood pressure is due to
cryoblockade only in the targeted region, and it uses
similar cooling gradients in the brains of both experi-
mental and control groups.

Single midline placements were selected in some
rats because the cryoprobe could be positioned in the
ventricular space to touch and thus cool the bilateral
surfaces of the hypothalamus (AV3V region) or corti-
tal targets, thus precluding physical damage to the
tissue by the cryoprobe. This strategy is possible be-
cause the thermal conductivity of cerebrospinal fluid
(CSF) is approximately the same as brain tissue and
because CSF flow is slow. As a result, the cooling
gradients centered around intraventricular cryoprobes
are not irregular at the CSF-tissue interface.

After 1 week of recovery from the cryoprobe im-
plantation, each control and hypertensive rat was again
brieﬂy anesthetized with methoxyflurane and a cath-
eter was implanted in a peripheral artery (i.e., carotid
or femoral). The rat was then allowed 2 to 4 hours to
recover from the anesthetic. Control arterial pressure
was monitored for 30 minutes, and then the cryoprobe
was cooled to 5°C and maintained for at least 10 min-
utes. The cryoprobe temperature was subsequently
lowered to 0°C for at least 10 minutes and then turned
off to allow the surrounding tissue to rewarm to body
temperature. Blood pressure and heart rate measure-
ments were determined at the end of each control and
cooling interval. The two cryoprobe temperatures
were selected based on measurements of the spatial
temperature gradients.

Spatial temperature gradients were measured in the
tissue of the hypothalamus in a separate group of light-
ly anesthetized rats. The cryoprobe was stereotaxically
positioned in the midline in the third ventricle, with the
tip just above the optic chiasm. Different gradients
were produced by cooling the cryoprobe to different
temperatures. Each gradient was allowed to stabil-
ize for at least 10 minutes. The tissue temperature was
measured with the smallest-diameter copper-constan-
tan thermocouple manufactured (0.025 mm diameter;
Omega Engineering, Stamford, CT, USA). This size
was selected to minimize the thermocouple measure-
ment error produced by heat conduction along the ther-
mcouple wires. The thermocouple was attached to the
tip of a glass micropipette with Eastman 910 cement
(Rochester, NY, USA). Temperatures were measured
relative to a reference junction in ice water.

The thermocouple was inserted into the brain with a
stereotaxic instrument at a 45- or 90-degree angle rela-
tive to the cryoprobe. Approaches were made in both
the coronal and sagittal planes. Temperatures were
observed after 0.5-mm steps along the orthogonal tra-
jectory (0.707-mm steps for the 45-degree trajectory),
and at least 10 seconds was allowed for temperature
stabilization at each location. Measurements were
made only during the penetration of the thermocouple into the tissue. Several measurement tracks were made in each brain, but all tracks were separated by at least 3 mm of tissue.

At the termination of each experiment, the animal was killed (pentobarbital overdose) and the brain was fixed (formalin perfusion) and cut (frozen sections) so that serial reconstructions could be made to locate the cryoprobe position precisely. Control and targeted cryoprobe reconstructions, in combination with the observed cryoblockade effects on blood pressure, enabled statistical analyses to be made to determine the intracerebral locations of cooling gradients that reduced blood pressure elevations. T tests were used to determine mean differences in blood pressure between groups with different cryoprobe locations. In the case of repeated samples, data were evaluated with analysis of variance followed by Tukey's test to determine significant groups. To validate the homogeneity of variance assumption, the Hartley $F_{max}$ test was used.

**Results**

Figure 1 shows the temperature gradient in the coronal plane measured during a 45-degree penetration of the thermocouple into the hypothalamus of a typical rat. Continuous measurements of cryoprobe and tissue temperatures showed stable values after 3 minutes of cooling. All thermocouple measurements indicated cylindrical gradients surrounding the cryoprobe (i.e., oval in cross-section, given the 0.5 x 1-mm shape of the probe), the spatial dimensions of which were proportional to the temperature of the cryoprobe's surface.

For an approach of the thermocouple tip toward the surface of the 5°C cryoprobe (i.e., toward the rounded, 0.5-mm diameter surface), it can be observed (see Figure 1) that when the temperature of the cryoprobe was at 5°C, the preoptic hypothalamus, out to 2.5 mm, was between 20 and 10°C. When the cryoprobe surface temperature was reduced to 0°C, the temperature gradient was between 10 and 0°C in this same hypothalamic tissue. For an anterior or posterior approach toward the surface of the 5°C cryoprobe (i.e., toward the rounded, 0.5-mm diameter surface), the 20°C point of the gradient was only 1.2 mm from the probe surface.

Figure 2 shows the effects on blood pressure produced by cooling cryoprobes in various cerebral locations for groups of both normotensive and DOCA-salt hypertensive rats. Hypothalamic temperature gradients between 20 and 10°C (i.e., cryoprobe at 5°C) had no effect on mean arterial pressure in either hypertensive (AV3V-H in Figure 2; precooling value, 189 ± 9 mm Hg) or normotensive (AV3V-N in Figure 2; 128 ± 2 mm Hg) rats; changing the gradient to the 10 to 0°C range (i.e., cryoprobe at 0°C) markedly reduced blood pressure, but only in the hypertensive rats.

The labeling of the hypothalamic group as AV3V is arbitrary, since cryoblockade of other structures also occurred. Clearly, not all hypothalamic cryoprobes were found adjacent to the AV3V location, but all hypothalamic temperature gradients produced by 0°C cooling reduced temperatures in AV3V below 10°C. Similar temperature gradients from cryoprobes just dorsal to the hypothalamus (THAL-H in Figure 2; 191 ± 1 mm Hg) or on the surface of the dorsomedial prefrontal cortex (173 ± 7 mm Hg) had no effect on mean arterial pressure in hypertensive rats. These two gradients, when applied to the ventromedial prefrontal cortex, however, reduced blood pressure in hyperten-

**Figure 1.** Spatial temperature gradients in the hypothalamus of the rat produced by cooling a midline cryoprobe to 5°C and to 0°C. Cooling of the probe to 5°C produced a 2.5-mm gradient in the hypothalamus that ranged from 10 to 20°C. This temperature range has been determined to block synaptic activity without altering axonal propagation. Cooling the probe to 0°C produced a 10 to 0°C gradient in the same hypothalamic region. This temperature range blocks both synaptic and axonal transmission.

**Figure 2.** Arterial pressure responses to cryoblockade at 5°C and 0°C in several regions of the brain of DOCA-salt hypertensive rats. All values are mean ± SE. AV3V = cryoblockade in the anteroventral hypothalamus near the third ventricle and other nearby hypothalamic structures; VMFC = cryoblockade in the ventromedial frontal cortex; THAL = cryoblockade in the thalamus just dorsal to the AV3V regions; N = normotensive rats; H = hypertensive rats (baseline blood pressure values are given in the text). Asterisk indicates statistically significant difference (AV3V, p<0.01; VMFC, p<0.05).
sive (VMFC-H in Figure 2; 194 ± 6 mm Hg), but not normotensive (VMFC-N in Figure 2; 132 ± 6 mm Hg), rats. Similar effects of the specific intracerebral temperature gradients on blood pressure elevations were observed in a second model of hypertension ( renovascular). These results are shown in Table 1.

Table 2 shows the effects of intracerebral cryoblockade on heart rate in two models of hypertension (DOCA-salt and renovascular). Note that the statistically significant reductions in blood pressure seen in Figure 2 are associated with parallel reductions in heart rate. These reductions, however, occurred only in the hypertensive rats. During cryoblockade, neither the normotensive nor the hypertensive animals manifested piloerection, a reaction associated with the thermoregulatory response.

Figure 3 shows the reconstructed locations of the effective temperature gradients that reduced blood pressure elevations in all of the hypertensive rats. Noneffective control placements of the cryoprobes (hatched) are matched with the targeted ones (stippled) to determine the boundaries of the effective gradients (boxes). All of the placements illustrated were reconstructed to the nearest 0.5-mm plane following histological examination. Each cryoprobe illustrated represents several overlapping ones from replicated experiments. The boxes designate the cooled brain regions that effectively reduced blood pressure.

**Discussion**

When the cryoprobe placed in the third ventricle is cooled to 5°C and then to 0°C, two distinct temperature gradients are produced in the hypothalamus (i.e., 20–10°C/2.5 mm and 10–0°C/2.5 mm, respectively). Studies by Benita and Conde[13] support the general conclusion drawn in the review by Brooks[7] that the synaptic transmission of neuronal elements contained within a 20 to 10°C temperature gradient is blocked without alteration of axonal propagation. Reducing the same spatially distributed temperatures to form a 10 to 0°C gradient produces blockade of the fibers of passage as well. As long as the cryoprobe does not go below −10°C, the blockade effects are completely reversible.[7,8-11,13] By inference, the blocking effect can be attributed to either synaptic dysfunction or lack of axonal propagation.

Van der Kooy et al.[6] demonstrated histochemically in the rat that extensive anterograde projections travel from the dorsomedial and ventromedial prefrontal cortices through the hypothalamus to the brainstem. Beckstead[5] has shown similar orthograde projections but from smaller injection sites limited to the dorsomedial prefrontal cortex. By comparing these two anatomical studies, it can be deduced that the projection sites in the medullary brainstem that are known to regulate blood pressure[14] are the exclusive targets of the ventromedial prefrontal cortex.

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**TABLE 1. Effects of Intracerebral Hypothalamic Cryoblockade on Arterial Blood Pressure in a Renovascular Model of Hypertension and in Normotensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain region</th>
<th>37°C</th>
<th>5°C</th>
<th>0°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renovascular HT (n = 5)</td>
<td>AV3V</td>
<td>148 ± 4</td>
<td>156 ± 8</td>
<td>136 ± 6*</td>
<td>150 ± 5</td>
</tr>
<tr>
<td>Normotensive (n = 5)</td>
<td>AV3V</td>
<td>128 ± 2</td>
<td>131 ± 2</td>
<td>125 ± 4</td>
<td>127 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. HT = hypertensive; AV3V = anteroventral hypothalamus near the third ventricle. *p<0.05, compared with 37°C control.

**TABLE 2. Effects of Intracerebral Cryoblockade on Heart Rate in Experimental Models of Hypertension and in Normotensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain region</th>
<th>37°C</th>
<th>5°C</th>
<th>0°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA (n = 5)</td>
<td>AV3V</td>
<td>418 ± 26</td>
<td>407 ± 29</td>
<td>292 ± 22*</td>
<td>370 ± 50</td>
</tr>
<tr>
<td>DOCA (n = 4)</td>
<td>VMFC</td>
<td>433 ± 35</td>
<td>383 ± 28*</td>
<td>358 ± 43*</td>
<td>484 ± 40</td>
</tr>
<tr>
<td>DOCA (n = 3)</td>
<td>THAL</td>
<td>450 ± 30</td>
<td>441 ± 23</td>
<td>432 ± 25</td>
<td>410 ± 20</td>
</tr>
<tr>
<td>DOCA (n = 4)</td>
<td>DMFC</td>
<td>486 ± 11</td>
<td>487 ± 17</td>
<td>480 ± 17</td>
<td>484 ± 20</td>
</tr>
<tr>
<td>Renovascular HT (n = 5)</td>
<td>AV3V</td>
<td>441 ± 17</td>
<td>417 ± 7</td>
<td>300 ± 19*</td>
<td>372 ± 15</td>
</tr>
<tr>
<td>Normotensive (n = 5)</td>
<td>AV3V</td>
<td>441 ± 19</td>
<td>446 ± 18</td>
<td>431 ± 34</td>
<td>438 ± 12</td>
</tr>
<tr>
<td>Normotensive (n = 2)</td>
<td>VMFC</td>
<td>490 ± 10</td>
<td>440 ± 20</td>
<td>390 ± 20</td>
<td>380 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE. HT = hypertensive.

The brain regions blocked include anteroventral hypothalamus adjacent to the third ventricle and other nearby hypothalamic structures (AV3V), thalamus overlying the AV3V regions (THAL), dorsomedial frontal cortex (DMFC), and ventromedial frontal cortex (VMFC). Blood pressure elevations in the experimental models were produced by subcutaneous DOCA-salt diet or renovascular insufficiency. *p<0.01, compared with 37°C control.
**Figure 3.** Anatomical reconstruction of the locations of all cryoprobes and extent of effective temperature gradients. Upper panel. Cryoprobe locations that were not effective in reducing blood pressure elevations in hypertensive rats (hatching) or produced statistically significant reductions (stippling) when cooled to 0°C; the boundaries of the effective temperature gradients (10-0°C, each of which blocks synaptic and axonal transmission) are indicated spatially (boxes). Lower panel. Location of the same cryoprobes as in the upper panel that were effective in reducing blood pressure elevations when cooled only to 5°C; the boundaries of the effective temperature gradients (20–10°C, each of which blocks only synaptic activities) are similarly indicated (boxes).

Structures (in uppercase letters): AA = area amygdaloid anterior; CAE = capsula externa; CAI = capsula interna; CO = chiasma opticum; CPF = cortex piriformis; F = columna fornix; FLC = fissura longitudinalis cerebri; FMP = fasciculus medialis proencephali (median forebrain bundle); FO = fornix; GP = globus pallidus; MI = massa intercalata; RCC = radiatio corporis callosi; SC = sulcus rhinalis; STP = stria terminalis pars precommissuralis; TCC = truncus corporis callosi; VL = ventriculus lateralis. Nuclei (in lowercase letters): cl = claustrum; cp = caudatus putamen; ha = anterior hypothalami; pom = preopticus medialis; pt = paratenialis; sc = suprachiasmaticus; sl = septi lateralis; so = septi supraopticus; st = interstitialis striae terminalis.

Our results show that cooling of the ventromedial prefrontal cortex to 5°C (i.e., at a location where synaptic activity would be expected to initiate activity in the frontocortical-brainstem neurons and at a temperature known to block synaptic activity) reduces blood pressure elevations and that cooling of only the hypothalamus to 0°C (i.e., at a location where axonal propagation in these neurons would be expected to occur and at a temperature known to block axonal propagation) produces the same antihypertensive effect.

The shape of the cryoprobe shaft is not symmetrical: it is longer in its anteroposterior direction (1 mm) than in its lateral dimension (0.5 mm). As a result, the temperature gradient extends 2.5 mm from the larger sides (as shown in Figure 1) and only 1.2 mm from the front and back. A frontal cortex cryoprobe (at 5°C) produces a tissue temperature gradient of 20 to 10°C that extends (from the center of the shaft) no more than 1.7 mm in the posterior direction. Thus, the boundaries of this temperature gradient would not impinge...
on the hypothalamus (AV3V region), 3 mm away. Therefore, our findings are not a result of inclusion of the AV3V within the effective cooling gradient.

The linearity of the gradient shown in Figure 1 was maintained as the thermocouple passed through the lateral ventricle, a finding that suggests that heat flow is not significantly altered by CSF. The nonlinearities in the temperature measurement that exist when the thermocouple is within 250 µm of the probe surface are presumed to be due to the establishment of significant temperature gradients within the metal of the thermocouple junction itself. This source of measurement error is minimized when the thermocouple junction is farther from the cryoprobe surface, because the two dissimilar metals are essentially at the same temperature.

Our present data support several conclusions. First, the reported effects of hypothalamic lesions on blood pressure in several models of hypertension are likely the result of destruction of fibers of passage originating elsewhere. Second, the fibers of passage appear to originate, in part, in the ventromedial prefrontal cortex, because blockade of synaptic function in that region partially reverses hypertension in the mineralocorticoid model of hypertension. Further cooling of the frontal cortex caused an additional fall in blood pressure. This additional reduction in blood pressure could be explained either by blockade of the axons in the area, by the expansion of the 20°C border of the gradient to block additional synapses, or by both. The finding that blood pressure was not completely normalized by blockade in the frontal cortex could be due to the failure of the largest temperature gradient created to reach all telencephalic neurons that send axons through the hypothalamus to the brainstem, especially those from the amygdala. Our general conclusion is that cerebral intervention in a frontocortical-brainstem system can reduce blood pressure elevations in models of hypertension with different etiologies (i.e., DOC A, renovascular).

Previous investigations in pigs have implicated a frontocortical-brainstem system in the initiation of lethal cardiac arrhythmogenesis. Intervention in this system by cryoblockade,15 β-adrenergic receptor antagonists,16 or behavioral habituation17 prevents the deleterious effects of psychosocial stress on the initiation of ventricular fibrillation in the acutely ischemic heart. If the same frontocortical-brainstem system regulates both arrhythmogenesis and hypertension, this would explain why hypertension is a risk factor for sudden cardiac death and why β-adrenergic receptor antagonists reduce blood pressure elevations, ventricular arrhythmias, and sudden death.18 In other words, both of these cardiovascular disorders are the result of activity initiated in the frontal lobes and projected to the brainstem cardioacceleratory centers.

References

Cryoblockade of the ventromedial frontal cortex reverses hypertension in the rat.
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