Effect of Serotonin Depletion on the Central Regulation of the Carotid Sinus Reflex in Rats

Akira Ito, M.D.* and Saul M. Schanberg, Ph.D., M.D.

SUMMARY

Effect of serotonin depletion in the rat brain on the sympathetic pressor response to occlusion of the common carotid arteries was examined along with the sequential transection of the brain stem. In rats with the brain serotonin depleted below 10% of control by p-chlorophenylalanine, the reflex pressor response significantly decreased, but the reflex sensitivity was unchanged in any transection regimen, suggesting the decrease originated in the associated hypertension. The infracollicular transection augmented the reflex reaction and sensitivity in both groups, and the medullospinal separation abolished the reflex. It is concluded that the brain serotonin would not be significantly involved in the central regulation of the sympathetic component of the carotid sinus reflex, and that the structure rostral to the midbrain could play an inhibitory role in the integrative regulation.

Additional Indexing Words:
- p-Chlorophenylalanine
- Norepinephrine
- Serotonin
- Carotid sinus reflex
- Brain stem transections

The biogenic amines including norepinephrine and serotonin are known to be concentrated in the brain stem where the reflex arc of the carotid sinus barofunction is located between the glossopharyngeal afference and both the vagal and sympathetic efferences. However, the roles of these supposed neurotransmitters in central regulation of the baroreflex remains obscure. While a few reports suggest a noradrenergic facilitation of the blood pressure and heart rate responses to altered sinus pressure, the role of serotonin which was found to elicit a significant contribution in the tonic blood pressure regulation by the brain stem is so far unknown.

The reflex elevation in blood pressure induced by occlusion of the common carotid arteries has been generally attributed to 1) suppression of the baroreceptors at the carotid sinuses; 2) anoxic excitation of the chemoreceptors at

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the carotid bodies; 3) possible ischemic state in the brain; and 4) hydro-
dynamic influence, among which the decreased afferent impulse in the
sinus nerves is signified as the most important mechanism because of the occlu-
sion effect being abolished after bilateral section of the sinus nerves.

The present study was, therefore, undertaken to examine the effects of
serotonin depletion in the rat brain stem induced by p-chlophenylalanine
(PCPA), a specific inhibitor of serotonin synthesis, on the sympathetic
component of the carotid sinus reflex.

Methods

Sprague-Dawley male rats, weighing 230-250 Gm, were used throughout steps
in the present study.

Either 100 mg of PCPA suspended in 1 ml of 0.9% saline or the vehicle was
injected intraperitoneally (i.p.) at 9 a.m. daily for 3 days. After 24 hrs of the last
injection, experimental and control, each 5, rats were decapitated under light ether
anesthesia. The brain was immediately picked out, and the brain stem, consisting
of the thalamus, hypothalamus, midbrain, pons and medulla oblongata, was sepa-
rated rostrally at the anterior commissure and the internal capsule and caudally at
the origin of the 1st cervical nerves.

Endogenous levels of norepinephrine (NE) and serotonin (5-HT) in the sample
were determined according to the method by Barchas et al with use of IRC-50
columns.

The carotid artery occlusion reflex was examined in remaining 11 rats, pre-
treated with either PCPA or saline. Rats were i.p. anesthetized with 60 mg/Kg of
α-chloralose and 500 mg/Kg of urethane, bilaterally vagotomized at the midcervical
portion, and were controlled the respiration at 60/min of the rate and 1.8-2.2 ml/
stroke of the tidal volume (Harvard Appar Co, Inc, Millis, Mass, USA). Mean
arterial pressure was recorded at the left femoral artery with an electric pressure-
transducer (P23Gb; Statham Instr Co, Hato Rey, Puerto Rico), and the heart
rate was calculated from the R-R interval of ECG (Lead II).

Reflex change in mean arterial pressure and heart rate were observed on a
complete occlusion of bilateral common carotid arteries for 25 sec which was repeated
for 3 times with intervals longer than 15 min and averaged.

The procedure was performed also after 45 min of transection of the caudal
brain stem when the blood pressure and the heart rate were both observed to settle
at new levels. The floor of the 4th ventricle was exposed by suction of the cere-
bellum in rats fixed stereotaxically (David Kopf Instr, Tujunga, Calif, USA), and
the brain stem was transected with a sharp thin spatula using a dissecting microscope
sequentially at the caudal border of inferior colliculi, separating the pons from the
midbrain, and at the caudal end of obex, seceding the medulla oblongata and the
spinal cord, according to topographical instructions (Fig. 1).

The reflex sensitivity in blood pressure was estimated on the regression coeffici-
ent in the correlation between the reactive elevation of blood pressure and the
basal level before occlusion, and on the highest level of blood pressure over which
the response was supposed to terminate on the regression equation.
 RESULTS

Levels of amines in the brain stem (Table I):
Following PCPA administration for 3 days, the level of 5-HT in the brain stem of rats decreased below 10% of the control, while that of NE remained unchanged.

Effects of the transection of the brain stem on the mean arterial pressure and the heart rate (Table II):
In association with 5-HT depletion in the brain stem, the mean arterial pressure elevated significantly, and remained almost unchanged after the infracollicular section but was eliminated by the medullospinal separation. Although the heart rate also increased by PCPA injections, it did not show any
Table I. Levels of Norepinephrine (NE) and Serotonin (5-HT) in the Brain Stem of Rats

<table>
<thead>
<tr>
<th>Regimen*</th>
<th>No. of Rats</th>
<th>Amine Level, ng/Gm wt. W**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NE</td>
</tr>
<tr>
<td>0.9% Saline, 1 ml</td>
<td>5</td>
<td>501.7±27.2</td>
</tr>
<tr>
<td>PCPA, 100 mg/Kg</td>
<td>5</td>
<td>538.9±13.8</td>
</tr>
</tbody>
</table>

* Pretreatment was performed for 3 days with either 1 ml of 0.9% saline or 100 mg/Kg of p-chlorophenylalanine (PCPA), i.p.

** The brain stem here consisted of thalamus to medulla oblongata extracted after 24 hrs of the last injection, and the amine levels in mean±S.E.M. were determined on Barchas et al.12)

° P<0.001, different from the level in the saline-treated.

Table II. Levels of Mean Arterial Pressure (MAP) and Heart Rate (HR) after Sequential Transections of the Brain Stem in Vagotomized Rats

<table>
<thead>
<tr>
<th>Transection*</th>
<th>Regimen** (No. of Rats)</th>
<th>MAP, mmHg</th>
<th>HR/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Saline (6)</td>
<td>107±4</td>
<td>370±12</td>
</tr>
<tr>
<td></td>
<td>PCPA (5)</td>
<td>137±8''</td>
<td>410±6'</td>
</tr>
<tr>
<td>Infracolliclar</td>
<td>Saline (5)</td>
<td>116±3</td>
<td>363±3</td>
</tr>
<tr>
<td></td>
<td>PCPA (4)</td>
<td>146±11'</td>
<td>372±24</td>
</tr>
<tr>
<td>Medullospinal</td>
<td>Saline (4)</td>
<td>45±3</td>
<td>296±7</td>
</tr>
<tr>
<td></td>
<td>PCPA (4)</td>
<td>51±5</td>
<td>321±16</td>
</tr>
</tbody>
</table>

* Sequential transection of the brain stem was performed in levels illustrated in Fig. 1 after 24 hrs of the last injection.

** Pretreated with either saline or PCPA as in Table I. Results are expressed as mean±S.E.M.

° P<0.05, °° P<0.01, different from the level in the saline-treated.

Table III. Reflex Responses in Mean Arterial Pressure and Heart Rate to the Carotid Artery Occlusion in Vagotomized Rats

<table>
<thead>
<tr>
<th>Transection</th>
<th>Regimen (No. of Rats)</th>
<th>Responses* in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAP, mmHg</td>
</tr>
<tr>
<td>None</td>
<td>Saline (6)</td>
<td>+25±2</td>
</tr>
<tr>
<td></td>
<td>PCPA (5)</td>
<td>+8±1***</td>
</tr>
<tr>
<td>Infracolliclar</td>
<td>Saline (5)</td>
<td>+33±3'</td>
</tr>
<tr>
<td></td>
<td>PCPA (4)</td>
<td>+18±2**,°°</td>
</tr>
<tr>
<td>Medullospinal</td>
<td>Saline (4)</td>
<td>+3±1</td>
</tr>
<tr>
<td></td>
<td>PCPA (4)</td>
<td>+2±1</td>
</tr>
</tbody>
</table>

* Response as mean±S.E.M. is an average of 3 times observations repeated with interval over 15 min. The common carotid arteries were occluded for 25 sec.

** P<0.01, *** P<0.001, different from the response in the saline-treated.

° P<0.05, °° P<0.01, different from the response before transection in each group.
consistent alteration on the surgical procedures in those vagotomized rats.

Reflex responses in the mean arterial pressure and the heart rate to the carotid artery occlusion (Tables III and IV, Fig. 2):

The reflex increase in the mean arterial pressure in the control group with bilateral vagotomy further augmented by the infracollicular transection, whereas the rats treated with PCPA elicited a response apparently smaller than in the control. The reaction also increased after the first transection, but was still smaller than in the former.

The dissection of the medulla oblongata at the caudal end of obex abolished the reflex in both two groups.

The heart rate, however, revealed no significant reactions throughout the steps in those animals already bilaterally vagotomized (Table III).

In order to estimate the reflex sensitivity, relations of the response in the mean arterial pressure with the basal level before occlusion were studied under sequential transections of the brain stem. Until the surgical level reached at the caudal medulla, the reflex response in blood pressure showed a reverse correlation to the basal level, in which the two groups with or without 5-HT diminution appeared to belong to a same aggregation although the blood pressure levels in the control rats did not range widely. The relation disappeared after the medullospinal separation similarly in both groups (Fig. 2).

The reflexibility was also compared on the level of transection. The 2 groups, here, were calculated together, since no difference in the sensitivity was observed as above.

That is, the infracollicular seccesion elevated the highest blood pressure level, over which the response was supposed not to develop on the regression equation, from 170 mmHg to 206 mmHg, although no difference was detected in the regression coefficient before and after the separation (Table IV).

**Discussion**

Serotonin level of the brain stem in the present study decreased below 10% of the control differently from that of NE, as already reported following administration of PCPA owing to its selective inhibition of 5-HT synthesis at the rate-limiting step by tryptophan hydroxylase. In association, the blood pressure in rats with depleted 5-HT elevated significantly, which had been attributed to block of the possible inhibitory function of 5-HT in the tonic vasomotor regulation by the caudal brain stem. The inhibitory effect of 5-HT has been also observed in the spinal sympathetic reflexes, although the relation of the brain 5-HT with the carotid sinus reflex is so far unknown.
Table IV. Kinetic Constants in the Response-Level Relation*

<table>
<thead>
<tr>
<th>Transection</th>
<th>No. of Rats</th>
<th>Regression Coefficient**</th>
<th>Highest Reflexible Level of MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>11</td>
<td>$-0.3315 \pm 0.1156$</td>
<td>170</td>
</tr>
<tr>
<td>Infracollicular</td>
<td>9</td>
<td>$-0.2455 \pm 0.1100^*$</td>
<td>260</td>
</tr>
<tr>
<td>Medullospinal</td>
<td>8</td>
<td>$-0.0583 \pm 0.1252$</td>
<td></td>
</tr>
</tbody>
</table>

* Both saline- and PCPA-treated rats were estimated together in this table, since no difference was observed in Fig. 2 on the relation of the reflex pressor reaction with the basal level of mean arterial pressure between the 2 groups.

** Regression coefficient and *** highest reflexible level of MAP over which the reflex was supposed not to develop were estimated on the regression equation in the relation at Fig. 2.

' Not different from the constant in rats without the section.

Fig. 2. Correlation of the reflex elevation in mean arterial pressure to occlusion of the common carotid arteries with the basal level before procedure, in respect to the transection level in the brain stem of vagotomized rats. An open circle indicates a saline-treated rat and a closed a PCPA-administered.
In the present study, the rats were bilaterally vagotomized so that the reflex impulse from the brain stem was expected to run through the sympathetic efferences. The PCPA-treated animals showed a reflex pressor response to occlusion of the common carotid arteries, which is elicited mainly in the baroreflex to decreased sinus pressure, significantly smaller than in the control.

The baroreflex reaction is, however, known to be reduced in hypertensive state due to resetting of the reflex threshold to the raised level of blood pressure and also due to the decreased sensitivity of baroceptive afferent nerves. The response in hypertension is considered to be diminished according also to the limit of contractibility of arterial walls already constricted.

Further steps in the present study were performed to investigate if the diminished response could be related with altered responsiveness beyond the influence of blood pressure level. As shown in Fig. 2 in which the control and the experimental rats were plotted together, the pressor effect was observed to decline as the basal level of blood pressure increased. Although the level in the control group did not range widely, the 2 kinds of rats appeared to belong to a same aggregation in the reverse correlations at any level in the brain stem, suggesting that the eliminated response in the 5-HT depleted animals was not fully derived from the impairment of serotonergic function but rather from hypertension originated in relation with reduction of serotonergic vasomotor performance in the brain stem.

That is, under the present experimental conditions, it seems difficult to impute a serotonergic effect in the central regulation of the sympathetic component in the carotid sinus reflex, which is reportedly not directly connected with the tonic vasomotor projections in the brain stem.

Besides, the suprabulbar function remains uncertainly signified in the central regulation of the reflex.

The relation between the pressor reaction and the basal level of blood pressure was eliminated after the transection of medulla oblongata in the present study. This is consistent with location of the major mechanism presumed at area postrema lateral to the obex. However, the decerebration reportedly augments the reflex response in blood pressure, and also in the present study, the infracollicular separation of the pons and the midbrain enhanced the pressor reaction in both groups although it elevated blood pressure only insignificantly. And while the regression coefficient in the response-level relation did not differ by this transection, the highest reflexible level of blood pressure markedly increased. These results seem to suggest that the brain structure rostral to the midbrain could play an inhibitory role in the integrative regulation of the carotid sinus reflex.
REFERENCES