Negative Chronotropic Response to Norepinephrine*

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SUMMARY
The sinus node artery of 11 anesthetized mongrel dogs was perfused at a constant pressure of 100 mmHg. A relatively small dose of norepinephrine, 0.01 to 1.0μg, usually induced a positive chronotropic response when injected directly into the sinus node artery. Occasionally, however, a combination of a positive and a negative chronotropic response or only a negative chronotropic response was apparent. A larger dose of norepinephrine, 10 to 30μg, consistently induced a triphasic response: there was a brief positive chronotropic response, followed by a longer negative chronotropic response, and finally a sustained positive chronotropic response. The negative chronotropic response usually began before the systemic blood pressure became elevated as a consequence of the systemic effects of the norepinephrine. The negative chronotropic response was not influenced appreciably by bilateral vagotomy. However, it was completely blocked by a small dose of atropine, 1μg. These results suggest that norepinephrine releases acetylcholine from parasympathetic nerve terminals.

Additional Indexing Words:
Sinus node artery  Norepinephrine

In a previous study (Hashimoto and Chiba, 1968),1 it was observed that naturally occurring catecholamines injected into the sinus node artery of the dog occasionally produced a negative chronotropic response (NCR). It was also demonstrated that this NCR was blocked by treatment not only with atropine but also with phenoxybenzamine, hexamethonium, or tetrodotoxin (Hashimoto and Chiba, 1969;2 Hashimoto, Chiba, and Suzuki, 1970).3 This suggested that injected catecholamine acted at a presynaptic alpha-receptor to induce the ultimate release of acetylcholine from postganglionic vagal fibers.

In the present experiments, we attempted to determine whether nor-
epinephrine (NE) consistently evoked an NCR if administered in an appropriate dose.

METHODS

Eleven experiments were conducted on mongrel dogs which were anesthetized with morphine sulfate, 2 mg/Kg i.m., followed in 30 min by an intravenous injection of chloralose, 100 mg/Kg. The chest was opened at the right 4th intercostal space and artificial respiration was instituted. The pericardium was incised longitudinally and the heart was suspended in a pericardial cradle. The sinus node artery was cannulated and perfused at a constant pressure of 100 mmHg (Hashimoto et al., 1967, 1968). Perfusion pressure and systemic blood pressure were measured with Statham strain gauges. To record the electrical activity of the atria and ventricles, bipolar intracardiac catheters were introduced into the atrium via a small incision in the tip of the right auricular appendage and into the right ventricle via the right external jugular vein.

The atrial and ventricular electrograms and the arterial blood pressure were recorded on a Brush Mark 200 oscillograph and on a Honeywell tape recorder, model 7600. The PP interval was measured on a beat-by-beat basis by means of a parallel logic analog computer (EAI 580), and this interval was inscribed by the oscillographic recorder. The validity of the analog computer outputs was verified periodically by recording the atrial and ventricular electrograms at fast per speed. Sodium heparin, 500 units/Kg, was given intravenously at the beginning of the perfusion, and 200 units/Kg was added at 1 hour intervals.

The drug solutions, in a volume of 0.01 to 0.03 ml, were injected into the perfusion tubing near the cannula in the sinus node artery over a period of 4 sec by means of microinjectors (Hamilton Co). The drugs used in these experiments were norepinephrine bitartrate (Winthrop), propranolol hydrochloride (Ayerst), and atropine sulfate (Lilly).

RESULTS

A relatively small amount of NE, 0.01 to 0.03 μg, usually induced a slight positive chronotropic response (PCR) when injected into the sinus node artery. This PCR became greater with increasing doses of NE. Doses of 0.1 to 1.0 μg usually produced maximum acceleration of the sinus rate (PP interval = 250 to 280 msec) from an average control PP interval of about 350 msec. However, occasionally only NCR or a combination of PCR was apparent. With these dose levels of NE, those responses which included NCR assumed one of 3 patterns: (a) PCR was interrupted by NCR, (b) NCR was apparent before PCR, or (c) only NCR was observed. An example of the third type of response is shown in Fig. 1. A larger dose of NE, 3 μg, produced NCR in 5 of 9 experiments, and the systemic blood pressure was elevated by about 10 to 20 mmHg. A larger dose of NE, 10 to 30 μg, consistently induced a triphasic response:
Fig. 1. A negative chronotropic response to 1 μg of norepinephrine (NE) injected into the cannulated sinus node artery. Recordings from above down represent arterial blood pressure and PP interval. Top tracing is time, in seconds.

Fig. 2. Typical response patterns of the sinoatrial node to increasing doses of norepinephrine (NE) injected into the sinus node artery. The downward deflections of the timer at the top of the record indicate 10 sec intervals.

there was a brief PCR, followed by a longer NCR, after which there was a sustained PCR. The NCR usually began before the systemic blood pressure became elevated as a consequence of the systemic effects of the NE.

Fig. 2 shows the chronotropic responses to increasing doses of NE in a representative experiment. With doses of 0.1 and 1 μg, a monophasic PCR was observed, but triphasic responses were obtained with 3 and 10 μg. NCR was not prevented by bilateral cervical vagotomy, as shown in Table I. The NCR was occasionally smaller after vagotomy, but the difference was not
Table I. Effect of Bilateral Vagotomy on the Negative Chronotropic Response to 3-10 µg of Norepinephrine in Seven Preparations

<table>
<thead>
<tr>
<th>Control PP Interval (msec)</th>
<th>Maximum PP Interval after NE (msec)</th>
<th>Control PP Interval (msec)</th>
<th>Maximum PP Interval after NE (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>319±20*</td>
<td>455±36</td>
<td>314±16</td>
<td>398±35**</td>
</tr>
</tbody>
</table>

* Values are means ± SEM.
** Not significantly different from before vagotomy; p>0.1.

Table II. Responses to Norepinephrine (NE) Injected into the Sinus Node Artery

<table>
<thead>
<tr>
<th>Dose of NE (µg)</th>
<th>No. of Dogs</th>
<th>Control PP Interval just before NE (msec)</th>
<th>Minimum PP Interval after NE (msec)</th>
<th>Frequency of NCR to NE</th>
<th>Maximum PP Interval after NE (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>9</td>
<td>356±25*</td>
<td>305±17</td>
<td>1/9</td>
<td>358±24</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>355±25</td>
<td>293±14</td>
<td>2/9</td>
<td>424±47</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>346±22</td>
<td>285±6</td>
<td>5/9</td>
<td>418±21</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>362±20</td>
<td>295±7</td>
<td>8/9</td>
<td>456±15</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>355±15</td>
<td>296±10</td>
<td>3/3</td>
<td>406±34</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM.

significant.

Table II summarizes the effects of NE at doses of 0.1 to 30 µg administered into the cannulated sinus node artery. The threshold dose level of NE for inducing NCR was approximately 1 to 3 µg. With 10 to 30 µg, a prominent phase of NCR was almost invariably observed. Ten µg of NE produced NCR in 8 of 9 dogs, and 30 µg elicited NCR in all 3 dogs to which this dose was administered. One of these 3 animals was the dog in which 10 µg failed to evoke NCR. The NCR with 10 to 30 µg of NE was frequently so pronounced that AV nodal rhythm supervened temporarily, as indicated by retrograde VA conduction on the atrial and ventricular electrograms. Occasionally a large dose of NE induced atrial fibrillation following pronounced depression of sinus rate.

The NCR induced by large doses of NE was completely blocked by a small amount of atropine injected into the sinus node artery. In each of 4 experiments, the NCR induced by 10 µg of NE was completely inhibited by treatment with 1 µg of atropine.

A relatively small amount of propranolol, 1 µg, injected into the sinus node artery suppressed the PCR in response to smaller doses of NE, 0.01 to 0.1 µg. A larger dose of propranolol, 10 to 30 µg, usually caused a marked
depression of sinus rate, and an AV nodal rhythm occasionally occurred. Therefore, it was difficult to ascertain the blocking effect of propranolol on the NCR.

**DISCUSSION**

In a recent review of cardiac sympathetic-parasympathetic interactions, examples were cited in which the peripheral components of one division of the autonomic nervous system were excited as a consequence of activity in the other.\(^6\) In the dog heart, Katoh (1964)\(^7\) occasionally observed a deceleration of sinus rate by topical application of epinephrine and NE to the SA node area. He ascribed such a paradoxical response to ischemic depression of pacemaker activity caused by local vascular constriction. Hashimoto et al (1968,\(^1\) 1970\(^3\)) also observed similar paradoxical responses to injection of naturally occurring catecholamines into the sinus node artery. However, Chiba and Hashimoto (1970)\(^8\) showed that NE usually caused an increased flow rate in the sinus node artery, which tends to contradict the hypothesis of Katoh. Conceivably, however, there could still be decreased flow through SA node tissue, but disproportionately increased flow in the surrounding atrial muscle supplied by the sinus node artery. Therefore, the role of sinus node blood flow is still uncertain.

In this study, we observed a phase of marked deceleration of sinus rate in response to NE in all experiments, provided the dose was sufficiently large. This NCR was elicited even after bilateral vagotomy, which would eliminate the major efferent pathway for reflex sinus deceleration. Hence, the NCR would not be ascribed to levation of the systemic blood pressure which occurred when the inta-arterially injected NE reached the systemic circulation. The NCR was completely abolished by atropine. These results suggest that large doses of NE release a significant quantity of acetylcholine, probably from vagal terminals in the region of the S-A node.

Very large doses of NE were usually necessary to evoke the NCR in these experiments. Therefore, whether this response represents a valid physiological mechanism must be questioned. It is difficult to compute the maximum concentration of NE at vagal terminals in the S-A node after injection of a given quantity of NE into the sinus node artery. Also, it is difficult to compare such an estimated NE concentration at vagal terminals with that expected to occur as a consequence of a given level of cardiac sympathetic neural activity.

A very crude biological assay of such concentration was attempted in the present study by using the maximum increase in heart rate in response to intra-
arterially injected NE as an index. The large doses of NE used in these experiments produced a PCR (Table II) which would be expected to be produced by an average frequency of cardiac sympathetic neural stimulation of about 4 sec⁻¹, according to a previous study from this laboratory (Levy et al, 1969⁹). Such a frequency of neural activity is certainly within the physiological range. If the local concentration of NE at the vagal terminals is similar to that at the S-A nodal pacemaker cells after injection of NE into the S-A nodal artery, then perhaps the NE concentration at these vagal sites is also in the physiological range.

The study of innervated atrial preparations by Leaders (1963)¹⁰ lends support to the hypothesis that the NCR observed in the present experiments might represent a physiological mechanism. After cessation of sympathetic neural stimulation, Leaders observed a period of cardioinhibition which was augmented by physostigmine and abolished by atropine. Hence, it is likely that the NE concentration during cardiac sympathetic neural stimulation may attain concentrations at cardiac vagal terminals equivalent to those reached after injection of the large doses of NE used in the present series of experiments.

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REFERENCES