Study of the Relationship Between the Neurotransmitter Store and Adrenergic Nerve Block Induced by Reserpine and Guanethidine

By Thomas E. Gaffney, M.D., Charles A. Chidsey, M.D., and Eugene Braunwald, M.D.

It is now established that the administration of reserpine or guanethidine is capable of preventing the response of effector organs to direct stimulation of post-ganglionic sympathetic nerves, even though the effector organ system remains responsive to the administration of the adrenergic transmitter, norepinephrine. Since both of these drugs have been shown to be capable of producing depletion of the tissue contents of norepinephrine, it has been suggested that depletion of the adrenergic transmitter store is the fundamental mechanism by which reserpine and guanethidine block adrenergic transmission. However, McCubbin and associates have suggested, and Cass and Spriggs have offered strong indirect evidence to support the view that guanethidine blocks adrenergic transmission before it produces measurable tissue norepinephrine depletion. No information is available on the relationship between the degree of blockade to sympathetic stimulation produced by reserpine and guanethidine block adrenergic transmission. However, McCubbin and associates have suggested, and Cass and Spriggs have offered strong indirect evidence to support the view that guanethidine blocks adrenergic transmission before it produces measurable tissue norepinephrine depletion. No information is available on the relationship between the degree of blockade to sympathetic stimulation produced by reserpine and guanethidine block adrenergic transmission. It was the objective of the experiments described in this report to compare the mechanisms of the adrenergic blockade induced by reserpine and guanethidine. Particular attention was directed toward establishing a quantitative relationship between the size of the store of sympathetic transmitter, as reflected in the myocardial content of norepinephrine, and the degree of adrenergic nerve blockade produced by these two drugs.

From the Cardiology Branch, National Heart Institute, U. S. Public Health Service, Bethesda, Maryland.

Received for publication September 26, 1962.

Methods

The chronotropic response to cardioaccelerator nerve stimulation and the level of norepinephrine in the left atrial appendage were measured in four untreated control dogs, in 25 dogs following a single intravenous injection of 3 mg/kg of reserpine, and in five dogs following an intravenous injection of 10 mg/kg of guanethidine. Between five minutes and 12 hours after the intravenous injection of either reserpine, or guanethidine, the dogs were anesthetized with an intravenous injection of 15 to 30 mg/kg pentobarbital. The femoral artery was cannulated and arterial pressure was measured with a P23A Statham transducer. Following endotracheal intubation, respiration was maintained with a Harvard pump. The chest was opened through a median sternotomy incision; the right cardioaccelerator nerve was dissected free for approximately 1 to 1.5 cm, and was stimulated with a Grass stimulator and bipolar silver electrodes at a frequency of 10/sec for 30 to 45 seconds. Impulses were of one millisecond duration and supramaximal voltages, ranging from 6 to 14 volts, were used; the voltage was confirmed to be supramaximal by gradually increasing it until the heart rate no longer increased. When there was no response to cardioaccelerator nerve stimulation, the maximal voltage (14 volts) was employed, and the technics of nerve isolation and stimulation were identical to those utilized prior to treatment. A single determination of the heart rate response to cardioaccelerator nerve stimulation was carried out in each dog. The chronotropic response to accelerator nerve stimulation, as measured on the electrocardiogram, was determined as soon as possible after the thoracotomy, usually within 15 minutes following the injection of pentobarbital. In the five dogs which received guanethidine, nerve stimulation was carried out as soon as the tachycardia and pressor response resulting from this drug had subsided, i.e., 30 to 60 minutes following injection. In two of the control dogs, and in two of the dogs which received intravenous injections of 10 mg/kg guanethidine, the right ventricular contractile
TABLE 1
Atrial Norepinephrine Content μg/g. Levels of Norepinephrine in the Left Atrial Appendage of Dogs Before and 30 Minutes After the IV Injection of 10 mg/kg Guanethidine.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Control</th>
<th>30 min after guanethidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.54</td>
<td>2.61</td>
</tr>
<tr>
<td>2</td>
<td>2.89</td>
<td>2.71</td>
</tr>
<tr>
<td>3</td>
<td>3.00</td>
<td>3.12</td>
</tr>
<tr>
<td>4</td>
<td>2.91</td>
<td>2.88</td>
</tr>
<tr>
<td>5</td>
<td>2.77</td>
<td>2.83</td>
</tr>
</tbody>
</table>

force was estimated with a Walton-Brodie strain gauge arch before and during cardioaccelerator nerve stimulation.

As soon as the heart rate response to accelerator nerve stimulation was recorded, the left atrial appendage was removed and frozen immediately. The tissue samples were homogenized with trichloroacetic acid and the catecholamines in the tissue extract were determined spectrofluorometrically and expressed as norepinephrine equivalents. The standard deviation of the difference between duplicate determinations with this method was 0.11 μg/g, and the lower level of sensitivity of the method was 0.05 μg/g. In six additional dogs the right atrial appendage and area of the sino-auricular node were biopsied at various times following the intravenous injection of 3 mg/kg of reserpine and the norepinephrine content of these tissues was measured.

Following the abolition of the chronotropic response to cardioaccelerator nerve stimulation after the administration of reserpine to five dogs and of guanethidine to five dogs, an intravenous infusion of 1-norepinephrine bitartrate was given at a rate of 5 μg/kg per min for 30 minutes. This was done in an attempt to replete or augment the adrenergic transmitter store, and thereby to restore the heart rate response to cardioaccelerator nerve stimulation. The latter was tested 15 minutes after discontinuation of the norepinephrine infusion. The response to nerve stimulation was also determined after the same dose of norepinephrine had been administered to four dogs which had received 10 mg/kg guanethidine intravenously 48 hours earlier, and to six dogs which had been pretreated with reserpine, 0.1 mg/kg injected intraperitoneally on each of two successive days prior to the experiment. In two other dogs pretreated with reserpine in this manner, 50 μg/kg per min of dopamine was infused for 30 minutes. The response to cardioaccelerator nerve stimulation was determined 15 minutes after completion of this infusion. In addition, atrial norepinephrine levels were measured before and 15 minutes after the discontinuation of the dopamine infusion in these dogs.

Results

GUANETHIDINE
Guanethidine, 10 mg/kg, completely abolished the positive chronotropic and inotropic responses to supramaximal, post-ganglionic cardioaccelerator nerve stimulation within 30 minutes after its intravenous injection (fig. 1), and this effect occurred prior to any measurable reduction in the norepinephrine content of atrial tissue (table 1). Prior to guanethidine administration the increase in heart rate resulting from cardioaccelerator nerve stimulation ranged between 43 and 93 beats/min. Thirty to 60 minutes after this drug the change in heart rate ranged between +2 and −4 beats/min. The adrenergic nerve blockade produced by guanethidine persisted for approximately three hours before measurable reduction of the atrial content of norepinephrine occurred. Infusion of 5 μg/kg per min of norepinephrine for 30 minutes at various intervals up to 48 hours following the injection of guanethidine failed to restore the
The relationship is shown between the level of norepinephrine in the left atrial appendages at the top of the figure and the heart rate response to right cardioaccelerator nerve stimulation at the bottom of the figure. The base of each vertical bar represents the control heart rate before stimulation. The peak of each bar represents the heart rate observed during supramaximal accelerator nerve stimulation at a frequency of 10 per second. The four observations on the far left were made in control dogs. The 25 observations to the right of the broken horizontal axis were each carried out in different dogs at various time intervals following the iv injection of 3 mg/kg reserpine. The five horizontal lines represent the heart rates in the dogs in which this rate was not altered during nerve stimulation. The concentration of norepinephrine in the left atrium of these dogs is represented by closed circles plotted immediately above the vertical bars or horizontal lines. The closed squares and closed, triangles represent the concentration of norepinephrine in the right atrial appendage and in the area of the sino-auricular node respectively. These specimens were obtained from animals in which the response to accelerator nerve stimulation was not tested.

The positive chronotropic response to accelerator nerve stimulation. Following the norepinephrine infusion cardioaccelerator nerve stimulation produced a change in heart rate which ranged between +1 and —4 beats/min.

Reserpine

The positive chronotropic response to cardioaccelerator nerve stimulation remained essentially unchanged until approximately five hours following the intravenous injection of 3 mg/kg of reserpine. During this time period, the left atrial norepinephrine content decreased from levels ranging from 2.5 to 3.0 μg/g, observed in the control dogs, to levels ranging from 0.2 to 0.9 μg/g. More than five hours following the single intravenous injection of 3 mg/kg reserpine, when the level of left atrial myocardial norepinephrine had decreased to below approximately 0.3 μg/g, the response to accelerator nerve stimulation fell to zero (fig. 2). Following the administration of reserpine, the norepinephrine concentrations in the right atrial appendage and in the area of the sino-auricular node were observed to fall at the same rate as in the left atrial appendage (fig. 2).

The infusion of norepinephrine did not restore the positive chronotropic response to cardioaccelerator nerve stimulation in five of the dogs in which it had been abolished by acute reserpinization. Following the norepinephrine infusion cardioaccelerator nerve stimulation produced a change in heart rate which ranged between +2 and 0 beats/min. In the six dogs which had been pretreated with 0.1 mg/kg reserpine on the two days prior to the experiment, the change in heart rate produced by cardioaccelerator nerve stimulation ranged between +1 and —2 beats/min. Following the norepinephrine infusion the change in heart rate ranged between +2 and —6 beats/min. The infusion of dopamine did not restore the positive chronotropic response to accelerator nerve stimulation in the two dogs in which this was tested. Prior to the infusion of dopamine the change in heart rate induced by nerve stimulation was +2 and —2 beats/min, while following the infusion it was 0 and +1 beats/min. However, the atrial concentration of norepinephrine was not increased by the dopamine; these levels were 0.14 and 0.02 μg/g prior to, and 0.03 and 0.04 μg/g after, the infusion.

Discussion

The blockade of the chronotropic response to cardioaccelerator nerve stimulation produced by guanethidine occurred before a measurable reduction of atrial catecholamine content occurred; this blockade was
not overcome by a prolonged infusion of a relatively large dose of norepinephrine. Hence, the interference with peripheral adrenergic transmission which followed the intravenous injection of guanethidine does not appear to be dependent upon depletion of the adrenergic transmitter store. This observation is direct evidence in confirmation of McCubbin et al. and Cass et al. who provided highly suggestive indirect evidence that the blockade of adrenergic transmission which occurs immediately after the injection of guanethidine is independent of norepinephrine depletion. However, the present experiments do not exclude the possibility that the reduction of tissue norepinephrine content occurs when the drug is used clinically and may be involved in the hypotensive effects of guanethidine.

In contrast to these effects of guanethidine, reserpine produced almost complete depletion of norepinephrine in both atrial appendages and in the area of the sinus node before any clear-cut effect upon the heart rate response to accelerator nerve stimulation was noted. The observation that the increase in heart rate produced by accelerator nerve stimulation remained unchanged despite a decrease in atrial norepinephrine content to a level of approximately 0.3 μg/g suggests that this concentration of norepinephrine approaches the critical concentration required for a positive chronotropic response to supra-maximal accelerator nerve stimulation. These data do not define an exact quantitative relationship between the size of the myocardial norepinephrine store below this level and the increment in heart rate produced by sympathetic nerve stimulation because of the limited accuracy of the analytic method. However, it appeared that as the tissue norepinephrine level decreased from approximately 0.3 μg/g to below 0.05 μg/g, the heart rate response to accelerator nerve stimulation was progressively reduced to zero. This finding suggests that the sympathetic nerve blockade induced by reserpine ultimately depends upon essentially complete depletion of the adrenergic transmitter store, regardless of whether the entire or only a portion of the norepinephrine store is available for release by nerve stimulation.

The observation that an infusion of norepinephrine did not overcome the adrenergic nerve blockade produced by reserpine was surprising in view of the report by Burn and Rand that the administration of norepinephrine to reserpine-pretrated cats restored the pressor response to direct sympathetic nerve stimulation and the observations of Gillespie and Mackenna that norepinephrine reversed the adrenergic nerve blockade produced by reserpine in the isolated rabbit intestine. The same dose of norepinephrine which was used in the present experiments had previously been shown to be capable of raising the atrial norepinephrine level in reserpine pretreated dogs from less than 0.05 μg/g to levels of approximately 1 μg/g at the time the response to cardioaccelerator nerve stimulation was tested, i.e., 15 minutes after the norepinephrine infusion was completed. This concentration (1 μg/g) is approximately four times as great as the minimal level of atrial norepinephrine associated with effective adrenergic transmission in the present experiments. The failure of this norepinephrine infusion to overcome the adrenergic nerve blockade produced by reserpine suggests that the norepinephrine which is deposited in the cardiac tissue of reserpine treated dogs is unavailable for release in response to stimulation of post-ganglionic adrenergic neurons. In contrast, an infusion of norepinephrine is known to restore the cardiac effects of tyramine in the reserpine treated animal and hence, to be available to tyramine for release. These observations, when viewed collectively, suggest that there may be two or more storage sites available for norepinephrine within the heart.

It seemed possible that repletion of tissue levels by infusion of a precursor to norepinephrine, dopamine, could restore the response to nerve stimulation. It has been shown that in rats, in which depletion was achieved with alph-methyl-meta-tyrosine, dopamine was capable of elevating the norepinephrine con-
centration in the heart. However, in these experiments the infusion of large doses of dopamine failed to produce a substantial increase in atrial norepinephrine content, and did not restore the heart rate response to accelerator nerve stimulation.

**Summary**

The heart rate response to cardioaccelerator nerve stimulation and the corresponding levels of myocardial norepinephrine content were determined and correlated in dogs at various time intervals following the intravenous injection of either 3 mg/kg reserpine, or 10 mg/kg guanethidine. Guanethidine produced complete blockade of the cardiac accelerator response before producing measurable myocardial depletion of norepinephrine. In contrast, reserpine reduced the positive chronotropic response to cardioaccelerator nerve stimulation only after myocardial norepinephrine levels have been reduced to approximately 0.3 µg/g. An infusion of norepinephrine did not restore the heart rate response to cardioaccelerator nerve stimulation in either the reserpine or guanethidine treated dogs. These data suggest that the interference with adrenergic transmission produced by guanethidine is independent of changes in the level of stored adrenergic transmitter. The reserpine-induced blockade of adrenergic transmission may ultimately be dependent upon the depletion of adrenergic transmitter, but almost complete depletion of stored adrenergic transmitter must occur before reserpine-induced adrenergic blockade occurs.

**References**


Study of the Relationship Between the Neurotransmitter Store and Adrenergic Nerve Block

Induced by Reserpine and Guanethidine

THOMAS E. GAFFNEY, CHARLES A. CHIDSEY and EUGENE BRAUNWALD

Circ Res. 1963;12:264-268
doi: 10.1161/01.RES.12.3.264

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1963 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/12/3/264

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/