Selective Activation of Noradrenaline and Adrenaline Producing Cells in the Cat’s Adrenal Gland by Hypothalamic Stimulation

By B. Folkow and U. S. von Euler

By stimulating different parts of the hypothalamus in the cat, it has been possible to show that adrenal medullary secretion often contains preferentially adrenaline or noradrenaline. The results indicate that the two hormones are secreted from different cells with separate innervation.

BRÜCKE, Kaindl and Mayer showed in 1952 that hypothalamic stimulation in the cat increased the adrenaline percentage of catechol amines in adrenal venous blood from about 11 per cent in the “resting” secretion to about 50 per cent. Since there is no consistent alteration in the adrenaline percentage during the increased secretion following carotid occlusion in the cat, the results of Brücke and associates seemed to indicate a selective stimulation of adrenaline secretion.

Acting on the assumption that the two medullary hormones could be selectively secreted, Euler and Folkow determined the catechol amines in the adrenal venous plasma in the cat under different kinds of reflex stimulation. Carotid occlusion again was noted to cause an increased secretion containing a low percentage of adrenaline, while sciatic or brachial plexus afferent stimulation caused a significantly larger proportion of adrenaline in the secretion. Although there was considerable scattering of the data, the adrenaline percentage during carotid occlusion was always lower than that obtained during sciatic or brachial plexus stimulation in the same animal. The relation of the adrenaline percentage figures during the two kinds of stimulation was 57 (±4.2):100.

In order further to investigate the possibility of inducing a selective secretion of noradrenaline or adrenaline, the relative and absolute content of the two hormones in the adrenal venous blood was studied during hypothalamic stimulation. Preliminary experiments showed that while the adrenaline content was usually increased, large amounts of noradrenaline were also obtained occasionally, suggesting that a release of either hormone could be induced. A more systematic study of the effects of stimulation of different parts of the hypothalamus was therefore undertaken.

METHODS

Cats were anesthetized with 0.05 Gm. chloralose per kilogram, and usually some urethane in addition. Electrodes, insulated except at the tip, were introduced into the hypothalamic region with the aid of a Horsley-Clarke instrument. The precise anatomic location of the electrode tip was determined after each experiment. In the majority of experiments, stimulation was effected with bipolar electrodes inserted at a distance of 2 to 3 mm. on each side of the midsagittal plane. The stimulation frequency was 60 per second with a voltage of 2 to 6 volts and a pulse duration of 5 milliseconds (square waves).

The collection of adrenal venous blood followed the procedure used by Brauner and colleagues. Blood (3 to 6 ml.) from the left adrenal was collected for one to four minutes and immediately centrifuged. The withdrawn blood was replaced by dextrane Tyrode solution. To prevent clotting the animal received heparin intravenously. Plasma samples were kept cold or frozen until analysis. After the electrodes had been placed in position, a control sample of adrenal venous blood was collected for determination of the “resting” secretion. Usually, in each experiment, stimuli were applied alternately with the bipolar electrodes placed in two previously selected positions. After two successive periods of stimulation, a control sample was
SECRETION OF ADRENALINE AND NORADRENALINE

Fig. 1. Diagram showing location of hypothalamic areas in which stimulation causes secretion of adrenaline and noradrenaline from the adrenals. Ch.O. = Chiasma opticum, C.a. = Commissura anterior, C.M. = Corpus mammillare.

again collected. Before taking each sample a few milliliters of blood were allowed to flow in order to wash out the tubing. On several occasions, 1 to 2 mg nicotine tartrate was injected intravenously at the end of the experiment. Hypothalamic stimulation was effective in 16 out of 17 experiments of this series.

The amounts of adrenaline and noradrenaline were determined by biologic assay using the cat's blood pressure and the chicken's rectal caecum. As shown by Dunér, cat's adrenal venous plasma can be used directly for assay purposes. Data are expressed in micrograms or millimicrograms per kilogram per minute from the left adrenal.

All of the 32 electrode positions have been recorded in figure 1.

In order to assess the relative secretion of noradrenaline and adrenaline, the data have been computed in the following way. The secretion of each hormone during stimulation has been related to the corresponding "resting" secretion and the relative increase for noradrenaline calculated according to the following expression, where each term is assumed to have a minimum value of 0; accordingly, the whole expression varies between 0 and 1.

$$\frac{\ln \frac{\text{HYP}(N)}{R(N)}}{\ln \frac{\text{HYP}(N)}{R(N)} + \ln \frac{\text{HYP}(A)}{R(A)}}$$

$\text{HYP}(N)$ and $\text{HYP}(A)$ represent secretion of noradrenaline and adrenaline, respectively, during hypothalamic stimulation, and $R(N)$ and $R(A)$ represent spontaneous (resting) secretion.

The relative increase in noradrenaline secretion has been expressed in figure 1 as the black part of the circle. Thus a white surface indicates increase in adrenaline secretion only (relative figures less than 0.1 have been put as 0). If increase in both secretions was less than 100 per cent, the result has been taken as zero (0).

The secretion data for the first electrode insertions only have been used as a basis for the above calculation. Generally, however, the mean figures for the relative increase in repeated insertions have deviated only little from the first ones, but occasionally deviation has occurred, possibly indicating disturbances due to hemorrhage or other reasons.

RESULTS

A. "Resting" Secretion. The "resting" secretion, which was determined in 15 of the 16 successful experiments, varied considerably; a certain and variable degree of reflex stimulation of the secretion is probably unavoidable in experiments of this kind. The figures for adrenaline were 0.0052 to 0.30 µg per kilogram per minute, with a mean figure of 0.063, and for noradrenaline 0.048 to 0.60 µg per kilogram per minute, with a mean of 0.20. A more even distribution was obtained on a log basis, giving mean figures of 0.038 and 0.16 µg per kilogram per minute of adrenaline and noradrenaline respectively. The spontaneous secretion figures showed a good agreement between each other in each single experiment. The average percentage of adrenaline in the spontaneous secretion in 15 experiments was 22 per cent (range 5.7 per cent to 57 per cent), which is similar to that found in previous investigations.

B. Effect of Hypothalamic Stimulation. The increase in adrenal secretion of both catecholamines was insignificant in one of the electrode positions in two animals (see fig. 1). In the remaining 30 electrode positions a definite increase in the secretion of either noradrenaline, adrenaline, or both was obtained. A selective increase of one or the other of the hormones as a result of stimulation may be assumed to have occurred in those cases where the increase of noradrenaline in relation to the total catecholamines (black portion of circle surface) was less than 0.3 or larger than 0.7. This occurred on 13 occasions. The most common effect was thus a fairly proportional increase in the secretion of both hormones.

Figure 1 gives a general survey of the results, showing the effect of stimulation in various electrode positions. Table 1 gives the figures from one complete experiment, and figures 2 and 3 illustrate the type of changes produced by shifting the electrodes.
Table 1 shows the large shifts in the proportion of secreted adrenaline when the stimulating electrodes were moved between two positions, P I and P II, without any appreciable alterations in the noradrenaline secretion. This is reflected in the adrenaline percentage figures which changed from 21 to 69 and from 18 to 76 when the stimulation point was altered from P II to P I, and from 66 to 26 when the electrodes were moved in the opposite direction. In this experiment, stimulation at P I and P II both increased noradrenaline secretion to about the same degree, whereas P I stimulation, in addition, selectively activated adrenaline secreting cells. Stimulation at P II increased the total secretion tenfold and more without significantly altering the proportion in comparison with the "basal" secretion. The stimulations were accompanied by a rise in blood pressure from 120 to 210-240 mm. Stimulation at P I caused hyperventilation and P II "shivering". The electrode positions were approximately the same as in experiment 27 (fig. 2).

Figures 2 and 3 demonstrate the shifts in secretion in experiments 27 and 32.

A selective activation of noradrenaline producing cells was obtained in experiments 21 and 25, where noradrenaline secretion increased from 0.14 to 0.48 and 0.048 to 0.53 μg per kilogram per minute respectively, while adrenaline secretion fell from 0.046 to 0.019 or increased (insignificantly) from 0.13 to 0.20 μg per kilogram per minute.

C. Effects of Nicotine. Nicotine was injected at the end of nine experiments in which the

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**Table 1.—Experiment 28, Cat 4.0 Kg., May 12, 1955**

<table>
<thead>
<tr>
<th>Adrenaline μg./ml</th>
<th>Noradrenaline μg./ml</th>
<th>Volume, ml</th>
<th>Time, min</th>
<th>Adrenaline μg./Kg./min.</th>
<th>Noradrenaline μg./Kg./min.</th>
<th>% Adrenaline</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.047</td>
<td>0.18</td>
<td>4.0</td>
<td>2.92</td>
<td>0.016</td>
<td>0.062</td>
<td>21</td>
<td>Spontaneous</td>
</tr>
<tr>
<td>0.24</td>
<td>0.88</td>
<td>4.0</td>
<td>2.5</td>
<td>0.096</td>
<td>0.35</td>
<td>21</td>
<td>P II</td>
</tr>
<tr>
<td>2.6</td>
<td>1.2</td>
<td>4.0</td>
<td>2.5</td>
<td>1.0</td>
<td>0.48</td>
<td>66</td>
<td>P I</td>
</tr>
<tr>
<td>0.001</td>
<td>0.25</td>
<td>4.0</td>
<td>3.33</td>
<td>0.027</td>
<td>0.075</td>
<td>27</td>
<td>Spontaneous</td>
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<tr>
<td>2.9</td>
<td>1.6</td>
<td>4.0</td>
<td>2.0</td>
<td>1.5</td>
<td>0.78</td>
<td>26</td>
<td>P I</td>
</tr>
<tr>
<td>0.52</td>
<td>1.5</td>
<td>4.0</td>
<td>1.92</td>
<td>0.27</td>
<td>0.78</td>
<td>17</td>
<td>Spontaneous</td>
</tr>
<tr>
<td>0.12</td>
<td>0.54</td>
<td>4.0</td>
<td>4.0</td>
<td>0.030</td>
<td>0.13</td>
<td>18</td>
<td>P II</td>
</tr>
<tr>
<td>0.58</td>
<td>2.7</td>
<td>4.0</td>
<td>2.67</td>
<td>0.22</td>
<td>1.0</td>
<td>76</td>
<td>P I</td>
</tr>
<tr>
<td>5.0</td>
<td>1.5</td>
<td>4.0</td>
<td>2.67</td>
<td>1.9</td>
<td>0.56</td>
<td>76</td>
<td>1 mg. nicotine</td>
</tr>
<tr>
<td>4.8</td>
<td>5.6</td>
<td>2.0</td>
<td>3.33</td>
<td>0.72</td>
<td>0.84</td>
<td>46</td>
<td>1 mg. nicotine</td>
</tr>
</tbody>
</table>
resting secretion was also determined. The results are shown in table 2.

The secretory effect of nicotine varied greatly as seen from table 2, not only with regard to the absolute figures but also in respect to the proportion of the two hormones. In experiment 30, in which a high adrenaline percentage (80 per cent) was observed with nicotine, unusually high adrenaline percentage figures were also noticed during stimulation in one of the two electrode positions.

Rapela and Houssay found a large increase in adrenaline and noradrenaline secretion from the adrenal gland of the dog after nicotine. An increase in the adrenaline percentage was noted in all cases, varying from 9 to 57 per cent (average 27 per cent), suggesting a mechanism by which adrenaline is secreted preferentially. In our material, the adrenaline percentage after nicotine was higher (average 17 per cent) than that of the nearest preceding spontaneous secretion in all cases except one.

DISCUSSION

Our experimental results confirm the findings of Brücke, Kaindl, and Mayer that hypothalamic stimulation may cause a large shift in the relative proportion of the two hormones released from the adrenal gland. While they found an increase in adrenaline only, we have obtained evidence, in some cases, for a selective stimulation of noradrenaline secretion also. The implication of these findings is clearly that each hormone must be secreted from specifically innervated cells. The physiologic proof of a dual secretory mechanism necessitates the assumption of differentiated cells producing one or the other of the two hormones. Recently, such an assumption has received strong support from the cytologic work of Hillarp and Hökfelt, who were able to stain noradrenaline selectively in groups of cells of the adrenal medulla.

Stimulation of the splanchnic nerve, or direct stimulation of cells with substances such as acetylcholine, nicotine, histamine or potassium, cannot be expected to reveal a selective secretory mechanism. Thus the experiments of Vogt and of Outschoorn show only that certain stimuli of the aforementioned type tended to stimulate both adrenaline and the noradrenaline secreting cells.

On the other hand, the splanchnic stimulation experiments of Büllbring and Burn did reveal a shift in the proportion of the secreted products which might be due to an alteration in the efficiency of nerves supplying one or the other type of cells during prolonged stimulation. The high adrenaline percentage in the secretion caused by efferent splanchnic stimulation in the experiments of Holtz and associates might be explained in a similar fashion.

There is little doubt that the selective activation or inhibition of the adrenaline-secreting cells caused by insulin hypoglycemia or by hyperglycemia can be explained along the same lines, that is, by the presence of a differentiated central representation of the secretory cell. The physiologic significance of these findings is also obvious. By an arrangement of this kind the organism is able to mobilize one or the other of the two hormones when needed. This would seem appropriate, for the difference in physiologic functions of the two hormones has been clearly borne out by recent observations on their circulatory and metabolic effects.

The results presented here also give some information as to the localization of the different hypothalamic areas that elicit noradrenaline and adrenaline secretion selectively or predominantly. Our data do not allow a close comparison with the functionally different areas studied by Bard and by Hess and their as-

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**Table 2.** Effect of Intravenous Nicotine on Secretion of Adrenaline (A) and Noradrenaline (NA) from the Cat's Left Adrenal Gland

<table>
<thead>
<tr>
<th>Exper. No.</th>
<th>Preceding &quot;resting&quot; secretion</th>
<th>% A</th>
<th>Dose of nicotine tartrate</th>
<th>Secretion after nicotine</th>
<th>% A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg./Kg./min.</td>
<td>mg./Kg.</td>
<td>% A</td>
<td>mg./Kg.</td>
<td>mg./Kg.</td>
</tr>
<tr>
<td>17</td>
<td>0.0052</td>
<td>0.061</td>
<td>1.0</td>
<td>1.0</td>
<td>0.63</td>
</tr>
<tr>
<td>19</td>
<td>0.021</td>
<td>0.14</td>
<td>13</td>
<td>1.0</td>
<td>3.2</td>
</tr>
<tr>
<td>25</td>
<td>0.19</td>
<td>0.27</td>
<td>41</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>27</td>
<td>0.008</td>
<td>0.21</td>
<td>32</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>28</td>
<td>0.030</td>
<td>0.13</td>
<td>17</td>
<td>2.0</td>
<td>0.72</td>
</tr>
<tr>
<td>29</td>
<td>0.18</td>
<td>0.41</td>
<td>31</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>30</td>
<td>0.30</td>
<td>0.58</td>
<td>34</td>
<td>1.0</td>
<td>5.6</td>
</tr>
<tr>
<td>31</td>
<td>0.0055</td>
<td>0.13</td>
<td>4.2</td>
<td>1.0</td>
<td>0.47</td>
</tr>
<tr>
<td>32</td>
<td>0.008</td>
<td>0.12</td>
<td>21</td>
<td>1.5</td>
<td>0.96</td>
</tr>
</tbody>
</table>
sociates, but it appears that the area associated with flight reactions is intimately concerned with adrenaline secretion.

Recent findings in the cat by Redgate and Gellhorn also seem to indicate a selective secretion of either adrenaline or noradrenaline into the peripheral blood depending upon intensity and location of the hypothalamic stimulation.

**SUMMARY**

Hypothalamic stimulation in the cat induced a secretion of adrenaline and noradrenaline from the adrenal gland in varying proportions depending on the location of the stimulus. From the selective activation of the secretion, it is inferred that adrenaline and noradrenaline producing cells are innervated by separate fibers with a different hypothalamic representation.

Nicotine increased the adrenaline percentage in the secretion in eight of nine cases, the average increasing from 22 to 39 per cent.

**REFERENCES**

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