THE EFFECT OF 5-HYDROXYTRYPTAMINE ON THE COTTON PELLET LOCAL INFLAMMATORY RESPONSE IN THE RAT*

BY JOSEPH R. BIANCHINE, M.D., AND NORMAN R. EADE, M.D.

(From the Department of Medicine, Division of Clinical Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland)

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Several clinical syndromes suggest that serotonin (5-HT) may be associated with the excessive formation of fibrotic tissue.

Several investigators consider the hyperserotoninemia of the carcinoid syndrome at least partially responsible for the peculiar, predominately right sided subendocardial and myocardial fibrosis often seen in this syndrome (1-8). Retroperitoneal fibrosis (2, 3, 8) and perivascular fibrosis (2) also have been described in patients with the carcinoid syndrome. Endomyocardial fibrosis in Uganda has been associated with the diet staple of bananas which contain high concentrations of 5-HT (9, 10). An association between 5-HT and fibrosis has also been considered to exist in scleroderma (11, 12) and arthritis (13).

Attempts at experimental stimulation of fibrosis by 5-HT have been inconclusive, however, chronic administration of 5-HT in different animal species by several investigators has failed to produce fibrosis similar to that of the carcinoid syndrome (4, 5, 14, 15). Chronic administration of 5-HT in the guinea pig, for example, failed to yield subendocardial fibrosis, although subcutaneous fibrosis did develop in these animals at the injection site after repeated daily injections of high doses of 5-HT (4, 15).

Experiments which suggest that drugs which deplete tissue 5-HT have antiinflammatory effects (16) lend support to the thesis. The finding that methysergide, a potent serotonin antagonist, may be associated with excessive amounts of fibrosis in certain patients with idiopathic retroperitoneal fibrosis is unexpected and difficult to explain (17).

Since agents which affect the early exudative phase of the initial inflammatory reaction alter the extent of subsequent fibroplasia (18), we have investigated the effect of 5-HT and certain related compounds on early inflammatory reactions using the cotton pellet granuloma technique of Meier (19) as modified by others (20-22).

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‡ Present Address: Departments of Medicine and Pharmacology, McGill University, Montreal, Canada.
Methods

Female albino Sprague Dawley rats (Carworth Farms, New City, N. Y.), weighing 100-150 g, were used throughout this study. Rats were given free access to water and routine laboratory food. They were lightly anesthetized with intraperitoneal pentobarbital sodium, the abdomen shaved clean, scrubbed with surgical soap, and washed with 70% alcohol. Two sterile cotton pellets weighing 27-30 mg ± 0.5 mg were individually implanted subcutaneously in the ventral abdominal area, well clear of the skin incision, which was then closed with stainless steel suture clips.

On the following day the animals were treated with either drugs or normal saline. All doses of drugs were calculated in terms of free base and were administered subcutaneously over the dorsal aspect of the hind limb, far away from the ventral implantation site of the cotton pellets. After either 2 or 7 days of therapy, the animals were again weighed and sacrificed with ether. The cotton pellets along with the surrounding inflammatory tissue were carefully dissected from the surrounding tissue. The pellets were dried overnight at 60°C before weighing. The net increase in pellet weight was determined by subtracting the tare weight of each individual pellet.

The adrenal and thymus glands were removed promptly, trimmed, and the wet weight determined immediately.

Adrenalectomized and sham-operated control Sprague Dawley rats were purchased from the Carworth Farms. In these animals cotton pellets were implanted 1 wk after adrenalectomy or sham operation. Adrenalectomized rats were given free access to saline drinking water throughout the period of observation.

RESULTS

The effect of serotonin (5-HT), 5-hydroxytryptophan (5-HTP), 5-hydroxyindolacetic acid (5-HIAA), and methysergide on the formation of granulomatous tissue around subcutaneously implanted sterile cotton pellets is summarized in Table I. Rats which received daily injections of normal saline (5 ml/kg body weight) served as controls. 5-HT (10 mg/kg body weight) administered subcutaneously each day for 7 days significantly inhibited the formation of granuloma tissue, mean of 80.1 mg ± 1.6 compared to the control mean of 42.5 mg ± 3.8 (P< 0.01). This inhibitory effect was easily visible at the time of removal of the implanted pellet, for the surrounding granulomatous tissue was thin, delicate, and pale compared with the control. Granulomata in rats receiving the lower dose of 5-HT (1 mg/kg) had normal appearance and dry weight.

Methysergide, a potent serotonin antagonist at the two dose levels studied, failed to alter granuloma weight significantly. However, methysergide (1 mg/kg) when administered 15 min prior to 5-HT (10 mg/kg) completely abolished the inhibitory activity of 5-HT on granuloma weight and appearance. As shown in Table I, 5-HIAA, a major metabolite of 5-HT, and 5-HTP, a metabolic precursor of 5-HT, failed to influence granuloma weight significantly. This granuloma-inhibiting effect of high doses of 5-HT does not support the thesis that 5-HT may cause fibrosis in certain situations.

The possibility of adrenal stimulation by serotonin was next studied by measuring the gross weight of the thymus and adrenal glands at the time of re-
moval of granulomas. The thymolytic effect of glucocorticoids is well recognized.

The effect of 5-HT and methysergide on granuloma, thymus, and adrenal weights is summarized in Table II. These observations were made at 2 and 7 days after implantation of the cotton pellets. Saline-treated rats served as con-

TABLE I
Effect of 7 Days' Treatment with Serotonin, Methysergide, 5-Hydroxyindolacetic Acid and 5-Hydroxytryptophan on Granuloma Weight Using the Cotton Pellet Technique

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Mean body wt change</th>
<th>Granuloma weight (dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (5 ml/kg)</td>
<td>10</td>
<td>+7.1</td>
<td>42.5 ± 3.8</td>
</tr>
<tr>
<td>5-HT (1 mg/kg)</td>
<td>10</td>
<td>+8.3</td>
<td>41.5 ± 2.9</td>
</tr>
<tr>
<td>5-HT (10 mg/kg)</td>
<td>9</td>
<td>+4.1</td>
<td>30.1 ± 1.6*</td>
</tr>
<tr>
<td>Methysergide (1 mg/kg)</td>
<td>10</td>
<td>+4.9</td>
<td>42.8 ± 3.1</td>
</tr>
<tr>
<td>Methysergide (10 mg/kg)</td>
<td>10</td>
<td>+5.2</td>
<td>38.7 ± 2.1</td>
</tr>
<tr>
<td>Methysergide (1 mg/kg) followed in 15 min by 5-HT (10 mg/kg)</td>
<td>10</td>
<td>+2.0</td>
<td>43.6 ± 4.1</td>
</tr>
<tr>
<td>5-HIAA (11 mg/kg)</td>
<td>9</td>
<td>+8.1</td>
<td>43.6 ± 3.3</td>
</tr>
<tr>
<td>5-Hydroxytryptophan (13 mg/kg)</td>
<td>9</td>
<td>+8.5</td>
<td>43.0 ± 3.2</td>
</tr>
</tbody>
</table>

* vs. saline—P < 0.01.

TABLE II
Effect of Serotonin and Methysergide on Granuloma, Adrenal, and Thymus Weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 days' treatment after pellet implantation</th>
<th>7 days' treatment after pellet implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Granuloma dry wt</td>
<td>Adrenal wet wt</td>
</tr>
<tr>
<td>Saline (5 ml/kg)</td>
<td>10</td>
<td>42.4 ± 1.3</td>
</tr>
<tr>
<td>Methysergide (1 mg/kg)</td>
<td>10</td>
<td>45.3 ± 1.5</td>
</tr>
<tr>
<td>5-HT (1 mg/kg)</td>
<td>10</td>
<td>42.3 ± 1.7</td>
</tr>
<tr>
<td>5-HT (10 mg/kg)</td>
<td>10</td>
<td>36.6 ± 1.7</td>
</tr>
<tr>
<td>Methysergide (1 mg/kg) followed by 5-HT (10 mg/kg)</td>
<td>10</td>
<td>41.5 ± 1.7</td>
</tr>
</tbody>
</table>

N, number of animals.

* vs. saline—P < 0.01.

† vs. saline—P < 0.05.

trols. Methysergide (1 mg/kg) and 5-HT (1 mg/kg) again did not significantly alter granuloma weight. In addition, thymus and adrenal weights were not significantly different from control in these rats.

The mean values for granulomas formed in rats after 2 and 7 days' treatment with 5-HT (10 mg/kg) were 36.6 mg ± 1.7 (P < 0.01) and 40.1 mg ± 1.4 (P < 0.05), respectively, as compared with the control granulomas at 2 and 7 days, whose means were 42.4 mg ± 1.3 and 45.4 mg ± 2.3 respectively. Control
thymus weight was 339.2 mg ± 20.9 at 2 days and decreased to a mean of 234.8 mg ± 21.7 \((P < 0.01)\) after 2 days of treatment with 5-HT (10 mg/kg), and to a mean of 211.8 mg ± 17.9 \((P < 0.01)\) after 7 days of treatment.

The mean adrenal weight after 2 days of saline injection was 50.7 mg ± 2.1. Adrenal weights increased to a mean of 58.3 mg ± 3.6 \((P < 0.05)\) after 2 days' administration of 5-HT (10 mg/kg), and to a mean of 58.0 mg ± 3.5 at 7 days.

Methysergide (1 mg/kg), administered subcutaneously 15 min prior to 5-HT

### TABLE III

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 days' treatment after pellet implantation</th>
<th>7 days' treatment after pellet implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Granuloma dry wt</td>
</tr>
<tr>
<td>Saline (5 ml/kg)</td>
<td>15</td>
<td>39.3 ± 1.5</td>
</tr>
<tr>
<td>5-HT (1 mg/kg)</td>
<td>10</td>
<td>40.1 ± 2.1</td>
</tr>
<tr>
<td>5-HT (10 mg/kg)</td>
<td>19</td>
<td>34.7 ± 1.7</td>
</tr>
</tbody>
</table>

N, number of animals.
* vs. saline—\(P < 0.05\).
\(\dagger\) vs. saline—\(P < 0.01\).

### TABLE IV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 days' treatment after pellet implantation</th>
<th>7 days' treatment after pellet implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Granuloma dry wt</td>
</tr>
<tr>
<td>Saline (5 ml/kg)</td>
<td>14</td>
<td>43.6 ± 1.1</td>
</tr>
<tr>
<td>5-HT (1 mg/kg)</td>
<td>10</td>
<td>40.0 ± 1.3</td>
</tr>
<tr>
<td>5-HT (10 mg/kg)</td>
<td>19</td>
<td>35.1 ± 1.7</td>
</tr>
</tbody>
</table>

N, number of animals.
* vs. saline—\(P < 0.01\).

(10 mg/kg), again blocked the granuloma-inhibiting effect of 5-HT and also blocked 5-HT changes in thymus and adrenal weights.

These studies confirm the observation that 5-HT induces hypertrophy of the adrenal gland (10, 12), which may then secrete higher levels of steroids, with resultant thymolytic and antiinflammatory activity. It is possible that the antiinflammatory effects of such endogenous glucocorticoids may mask any direct inflammatory or fibrosing effect of 5-HT. The possible masking effects of adrenal stimulation were eliminated by studying the effect of 5-HT in adrenalectomized rats. Sterile cotton pellets were implanted into adrenalectomized, sham-operated, and control rats in the manner described above, except that less peneto-
barbital was required for anesthesia in the adrenalectomized rats. In these experiments the pellets were implanted 1 wk after adrenalectomy or sham operation.

The results of these experiments are summarized in Tables III, IV, and V. In Table III, the thymolytic effect of 5-HT (10 mg/kg) is clearly evident after both 2 days and 7 days of administration. For example, after 7 days of this dose of serotonin, the thymus weight decreased from 318.0 mg ± 14.8 to 132 mg ± 5.1 ($P < 0.01$). 2 days and 7 days of treatment with 5-HT (10 mg/kg) again significantly inhibited the formation of granuloma tissue around the cotton pellet. This dose of 5-HT significantly increased the size of adrenal glands over control after 2 days of treatment but not after 7 days.

<table>
<thead>
<tr>
<th>TABLE V</th>
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</thead>
<tbody>
<tr>
<td><strong>Effect of 5-HT on Granuloma and Thymus Weights in Adrenalectomized Rats</strong></td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Saline (5 ml/kg)</td>
</tr>
<tr>
<td>5-HT (1 mg/kg)</td>
</tr>
<tr>
<td>5-HT (3 mg/kg)</td>
</tr>
<tr>
<td>5-HT (10 mg/kg)</td>
</tr>
</tbody>
</table>

N, number of animals.
* vs. saline—$P < 0.01$.

The ability of 5-HT (10 mg/kg) to inhibit granuloma weights or cause hypertrophy of adrenal glands in sham-operated animals is not as clearly interpreted as in the control groups of animals in Table III (see Table IV). For example, the mean control granuloma weight after 2 days of saline was 43.6 mg ± 1.1. After 2 days of treatment with 5-HT (10 mg/kg) the mean granuloma weight was 35.1 mg ± 1.2 ($P < 0.01$). Similarly, this dose of 5-HT significantly decreased the mean thymus weight to 290.1 mg ± 11.3 ($P < 0.01$) compared to the control mean of 363.2 mg ± 15.7. Despite the clear evidence that 5-HT had a thymolytic effect, the adrenal glands in sham-operated rats which received 5-HT (10 mg/kg), although heavier than control, were statistically different only at a level of $P < 0.05$.

In sham-adrenalectomized rats treated with 5-HT (10 mg/kg) for 7 days after pellet implantation, both granuloma and adrenal weights changed in the expected direction but not to a statistically significant degree. This dose of 5-HT did, however, significantly decrease the thymus weight.

The effects of 5-HT on granuloma and thymus weights in adrenalectomized
rats are summarized in Table V. An additional dose of 5-HT (3 mg/kg) was studied since adrenalectomized rats have been reported to be more sensitive to the toxic effects of 5-HT than control rats (23), but the adrenalectomized rats in the present study tolerated 5-HT up to and including 10 mg/kg well.

5-HT (1 mg/kg and 3 mg/kg) failed to alter significantly either granuloma weight or thymus weight from control levels. However, 5-HT (10 mg/kg) significantly increased the granuloma formation around the implanted sterile cotton pellet after both 2 and 7 days of therapy. For example, the mean granuloma weight in adrenalectomized rats treated with saline for 7 days was 59.0 mg ± 4.1, while the mean granuloma weight after 7 days of 5-HT (10 mg/kg) was 78.1 mg ± 4.1 (P < 0.01). In addition, it is interesting to note that adrenalectomy per se appears to stimulate granuloma formation, for the largest granulomata in saline-treated animals were seen in adrenalectomized rats.

DISCUSSION

The object of these studies was to determine if 5-HT enhances the inflammatory response to a standard stimulus. The cotton pellet technique of Meier (19) as modified by others (20-22) provides a quantitative measure of modifying factors in this inflammatory process. Since it is well known that factors which influence the extent of initial inflammatory reactions, may also regulate the extent of subsequent fibroplasia (18), we hope that studies of the effect of 5-HT on the early inflammatory response may provide a clue to 5-HT effect in fibroplasia or fibrosis occurring over prolonged periods.

The initial experiments revealed that 5-HT (10 mg/kg body weight) significantly inhibited the formation of inflammatory tissue reaction to the implanted cotton pellet rather than augmenting this response.

Winter (22), Steelman (21), and others have found that certain compounds which inhibit granuloma formation actually produce this effect indirectly by stimulating the adrenal gland to secrete antinflammatory glucocorticoids. Since these steroids also have thymolytic activity, they have found thymus weights to be a sensitive indicator of adrenal activity.

In the next series of experiments, the weights of the adrenal and thymus glands were recorded in addition to the granuloma tissue formed. 5-HT (10 mg/kg) again inhibited the formation of granuloma tissue after 2 and 7 days of treatment. However, this inhibitory effect appeared to be mediated via the adrenal axis, since significant atrophy of the thymus gland and adrenal hypertrophy were noted.

The effect of 5-HT on adrenal and other endocrine activities has been recently reviewed by Garattini (24). Both direct and indirect evidence strongly suggest that 5-HT stimulates the secretion of adrenocortical hormones (12, 25-27). Our data confirm in still another indirect manner that 5-HT stimulates the adrenal gland to hypertrophy and to secrete hormones with thymolytic activity. The precursor of 5-HT (5-hydroxytryptophan) is apparently ineffective in stimulating adrenal function (24). Our data agree with this finding, in that 5-hydroxytryptophan did not alter granuloma formation in the rat.
Franchimont and associates (28) found that 5-HT inhibited the formation of fluid exudate in the Selye granuloma pouch technique, while Georges and Herold (29) reported that 5-HT inhibited the edema reaction to dextran injections in the foot of the rat. Both these reports indicate that 5-HT has antiinflammatory effects, but the mechanisms of these effects were not investigated by either group. Penn and Ashford (20), using the cotton pellet technique coupled with a dye indicator method for measuring capillary permeability, found that the immediate increase (10–15 min) in capillary permeability which follows pellet implantation is inhibited by 5-HT.

Ashford and Penn (30) recently demonstrated that development of granulomata in adrenalectomized rats is inhibited if pellets were implanted on the day of adrenalectomy, but potentiated if pellets were implanted 7 days later. Our data confirm this augmentation effect of adrenalectomy on granuloma formation when cotton pellets are implanted 7 days after adrenalectomy. In addition, it is clear that 5-HT (10 mg/kg) further enhanced this granuloma formation.

Since 5-HT augments granuloma formation in adrenalectomized rats, it may conceivably increase later fibrosis. A major deterrent to speculation relating this finding in rats to fibrosing reactions in man is the very large doses of 5-HT required to produce these effects in the rat. The effective dose of 5-HT is well within the reported 120 in rats using subcutaneous routes (115 mg/kg) (23, 31, 32), although minimal level tubular dilatation has been noted when this dose is given over 10 days. Higher doses may cause significant renal damage (15, 33, 34). The only other rat tissue which is greatly influenced by the dose of 5-HT effective in the present study is the stomach, where mucosal lesions have been observed (33).

Another complicating factor is that adrenalectomized rats are more sensitive to the hypotensive and hypothermic effects of 5-HT. While these effects are marked at doses higher than those used in our experiment, moderate effects have been noted at 10 mg/kg (23). The adrenalectomized rats in the present study appeared grossly to tolerate 10 mg/kg as well as control rats.

Methysergide, a potent serotonin antagonist, has been associated in man with the development of idiopathic retroperitoneal fibrosis (17). Since this accumulation of fibrotic tissue in the retroperitoneal space may in some way be related to serotonin, we also studied the effect of methysergide on granuloma formation. Methysergide alone (1 mg/kg and 10 mg/kg) failed to alter granuloma formation. However, methysergide (1 mg/kg), when administered 15 min prior to 5-HT (10 mg/kg), prevented the inhibitory effect of 5-HT on adrenal secretion. These results thus provide no explanation for the possible relationship between methysergide and retroperitoneal fibrosis.

**SUMMARY**

The cotton pellet technique was used to evaluate the effect of 5-HT and certain related compounds on granuloma formation in the rat. 5-HT (10 mg/kg) significantly decreased granuloma formation, significantly increased adrenal...
weight, and significantly decreased thymus weight in normal rats, and significantly decreased granuloma formation and thymus weight in sham-operated rats. On the other hand, 5-HT (10 mg/kg) significantly increased granuloma weight in adrenalectomized rats.

Methysergide (1 mg/kg) blocked the inhibitory effect of 5-HT on granuloma formation as well as the changes in weight of adrenal and thymus glands.

5-HIAA (11 mg/kg) and 5-HTP (13 mg/kg) failed to alter granuloma formation.

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BIBLIOGRAPHY


