LONG-TERM EFFECTS OF INTRACEREBRAL CORTICOID IMPLANTS

By
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ABSTRACT

Chronic implantation of cortisol acetate in the basal medial hypothalamus resulted in a steady decrease in weight of the adrenal glands which remained severely atrophic up to 70 days post-implantation. At this time, however, the adrenal ascorbic acid depletion response to unilateral adrenalectomy was normal. The compensatory adrenal hyper trophy (CAH) response, which was inhibited in the immediate postoperative period, reappeared later, and had returned to normal by 21 days postoperatively. Intramuscular administration of cortisol in unimplanted rats inhibited CAH at 14 or 21 days following onset of treatment, and prevented the recovery of CAH in animals implanted 21 days previously with cortisol in the median eminence. The possibility is discussed that the differential recovery of the responses to unilateral adrenalectomy in implanted animals with continuing atrophy of the adrenal cortex is due to some adaptation of central nervous mechanisms subserving the CAH response.

In an earlier study on the effects of chronic hypothalamic implantation of cortisol (Chowers et al. 1963), an interesting dissociation was found to develop between two measures of pituitary-adrenal function: adrenal weight and ascorbic acid depletion in response to stress. While adrenals showed steadily increasing atrophy in animals with median eminence implants, the ascorbic acid response to unilateral adrenalectomy, which was inhibited during the first
two weeks following implantation, was restored in the third week. Other studies dealing with intracerebral corticoid implants have not focused on the development of changes in the pituitary-adrenal system as a function of time following implantation (see Davidson et al. 1967, for refs.). Since it appeared that these animals manifested certain characteristics of pituitary-adrenal function not readily obtainable by other experimental treatments, it was of interest to study the long-term changes in adrenal function in these animals resulting from the implants. Accordingly, a study was initiated on the relationship between three parameters of pituitary-adrenal function in rats with chronic implantation of crystalline cortisol: maintenance of adrenal weight in basal conditions, and the compensatory adrenal hypertrophy (CAH) and ascorbic acid depletion responses to unilateral adrenalectomy.

Recent investigations (Davidson et al. 1967) have indicated that small dexamethasone implants in certain extrahypothalamic regions of the forebrain, particularly the septum, inhibit CAH. To determine whether or not the phenomena under study were peculiar to median eminence implantation, the long term effects of dexamethasone implants in the septal region were also studied.

**MATERIALS AND METHODS**

Experiments were conducted on adult male rats of Wistar origin.

Implants of crystalline corticosteroids were stereotaxically placed in the median eminence region of the hypothalamus (as defined in Davidson & Feldman (1963) through stainless steel tubes which were fixed to the skull with dental cement and small screws, holding the implanted steroid in place. Cortisol acetate was implanted in the median eminence through 20-gauge tubes, pellets of approximately 200 μg of the steroid being ejected from the tube into the brain. The implants were left in place for varying periods of time up to 70 days. To control for such factors as mechanical trauma to the brain, cholesterol implants prepared in identical fashion were also placed in the median eminence and left *in situ* for 70 days. The effects of long-term steroid implantation on adrenal responses were compared with those in untreated rats. Dexamethasone was implanted either in the median eminence or the septal region, and in this case smaller tubes (24-gauge) were used. These contained approximately 30 μg of dexamethasone (free alcohol), and the steroid was not ejected from the tubes.

Injections of cortisol acetate in oil were performed intramuscularly, 6 days a week, for varying time durations, commencing either at the time of unilateral adrenalectomy or 7, 14 or 21 days before it, and terminating at autopsy. CAH was measured by comparing the weight of the right adrenal, removed either at the time of implantation or onset of injections, or at various time intervals thereafter, with that of the left adrenal removed 9–10 days later. As a measure of CAH, the adrenal weight difference (AWD) was used. This was calculated as

\[
\text{AWD} = \frac{(\text{left adrenal weight} - \text{right adrenal weight}) \times 100}{\text{right adrenal weight}}
\]

Adrenal ascorbic acid was determined by the method of Roe & Kuether (1943).

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To verify the location of implants in the median eminence region, the ventral surface of the brain was bared to allow visualization of the pellets or tips of the tubes; in the case of implantation in other brain regions, implants were located by studying serial sections of the brain in the region of the implant.

RESULTS

Adrenocortical responses following chronic hypothalamic implantation of cortisol

Fig. 1 shows adrenal weight in groups of rats sacrificed at different times following cortisol implantation. During the first four weeks adrenal weight declined steadily, and adrenals remained atrophic throughout the 68 days of observation. In Table 1, data on adrenal ascorbic acid and weight are shown for rats implanted 70 days previously with intrahypothalamic cortisol or cholesterol and for untreated controls. Despite the advanced state of adrenal atrophy, and the fact that ascorbic acid concentration in the adrenals was significantly depressed, unilateral adrenalectomy resulted in a significant depletion of adrenal ascorbic acid concentration. The percentage of adrenal ascorbic acid depletion was actually significantly greater in cortisol-implanted animals than in cholesterol-implanted controls. However, because of the decreased initial levels, the absolute concentration difference between left and right adrenals was not significantly changed by cortisol implantation. In none

Fig. 1.
Weights of both adrenals as a function of time following median eminence implantation of cortisol.
Table 1.
Adrenal weight and function 70 days following hypothalamic cortisol implantation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Adrenal weight (mg)</th>
<th>P</th>
<th>Right AAA* (µg/100 mg)</th>
<th>P</th>
<th>Right minus left AAA (µg/100 mg)</th>
<th>P</th>
<th>% depletion</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>32.8 ± 2.1</td>
<td>NS</td>
<td>614.2 ± 34.8</td>
<td>NS</td>
<td>217.5 ± 39.5</td>
<td>NS</td>
<td>35.6 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>15</td>
<td>34.1 ± 1.4</td>
<td>&lt;.001</td>
<td>594.9 ± 29.6</td>
<td>&lt;.001</td>
<td>192.7 ± 28.3</td>
<td>NS</td>
<td>30.9 ± 3.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cortisol</td>
<td>11</td>
<td>14.5 ± 1.3</td>
<td>395.0 ± 31.1</td>
<td>220.5 ± 20.2</td>
<td></td>
<td>56.7 ± 4.0</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adrenal ascorbic acid concentration.

of the measures was there any significant difference between untreated animals and those with intrahypothalamic cholesterol implants.

In a subsequent experiment (Fig. 2) the degrees of adrenal weight maintenance at intervals up to 33 days following cortisol implantation was studied together with the ability of the animal to respond to unilateral adrenalectomy with CAH. CAH is expressed as percentage AWD (see Materials and Methods). In Fig. 2 the open circles represent % AWD measured 9–10 days following removal of the right adrenal at the appropriate time shown by projection of the open circle to the x axis. It is seen that although in these animals adrenal weight continued to decrease for the duration of the experiment, % AWD, which was completely inhibited at the beginning of the experiment, showed a return to normal levels by the third week post-operatively*. A similar dichotomy between adrenal weight maintenance and CAH was produced by implantation of the more potent synthetic corticosteroid, dexamethasone. Small dexamethasone implants in the median eminence (24-gauge tubing, no ejection of the steroid pellet) produced, in 8 rats, a mean right adrenal weight of 7.8 ± 0.8** mg after three weeks and an AWD of 65.5 ± 17.4 %, 9 days thereafter. Nine rats with similar dexamethasone implants in the septal region, which showed similar atrophy of the right adrenal (7.6 ± 0.5 mg) also manifested significant CAH (AWD = 47.1 ± 20.2 %). Since the inhibition produced by septal implantation is less consistent than that resulting from hypothalamic implantation, these animals were selected from a larger group by

* In two previous studies from this laboratory, AWD's in untreated control rats under similar conditions were found to be respectively 62.7 ± 4.1 % (Davidson & Feldman 1963) and 76.7 ± 6.6 % (Feldman et al. 1966).

** Standard error of the mean.
Fig. 2.
Right adrenal weight and per cent adrenal weight difference (CAH) in rats at varying times following intrahypothalamic cortisol implantation. At each time interval the closed circles represent the weight of the right adrenal removed surgically at that time, and the open circles represent the percentage difference between the weight of the right adrenal removed at that time and the left adrenal removed 9–10 days later. Vertical bars are standard errors.

the criterion of having a right adrenal weight at least two standard deviations below the mean found in 333 normal rats***.

Systemic versus hypothalamic administration of cortisol
A comparison of the effects of various systemic doses of cortisol with that of a hypothalamic implant on CAH 9–10 days following unilateral adrenalectomy at the time of implantation or first injection is shown in Table 2. The effects of the implant could be roughly approximated by a daily injection of 1.5 mg cortisol. Fig. 3 shows that this dose, administered for periods up to

*** Right adrenal weight in 333 normal rats = 16.0 ± 0.2 mg.

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Table 2. Effects of hypothalamic or systemic administration of cortisol on compensatory adrenal hypertrophy 9–10 days following simultaneous unilateral adrenalectomy and onset of treatment.

<table>
<thead>
<tr>
<th>Systemic injection</th>
<th>N</th>
<th>Implant</th>
<th>% AWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/d</td>
<td>10</td>
<td>–</td>
<td>+13.8 ± 6.3</td>
</tr>
<tr>
<td>1.5 mg/d</td>
<td>11</td>
<td>–</td>
<td>+6.7 ± 6.8</td>
</tr>
<tr>
<td>–</td>
<td>15</td>
<td>+</td>
<td>+0.8 ± 6.9</td>
</tr>
<tr>
<td>2.0 mg</td>
<td>9</td>
<td>–</td>
<td>−21.4 ± 2.2</td>
</tr>
<tr>
<td>4.0 mg</td>
<td>10</td>
<td>–</td>
<td>−26.4 ± 4.1</td>
</tr>
</tbody>
</table>

Fig. 3. Right adrenal weight as a function of time following the onset of daily intramuscular administration of 1.5 mg cortisol acetate.

21 days, resulted in a steady decline of adrenal weight just as in the case of hypothalamic implantation. With regard to CAH, however, there was a considerable difference in the responses of systemically injected and hypothalamically implanted animals, at comparable time intervals following the
onset of treatment (Fig. 4). When right adrenals were removed 7 days after onset of treatment, there was complete inhibition of CAH in both cases and the AWD's were not significantly different. At 14 and 21 days, however, both the absolute weight difference between left and right adrenals and the $\%$ AWD were significantly lower in animals which received systemic injections of cortisol ($P < .01$).

To compare the sensitivity of systemically treated and hypothalamically implanted rats to superimposed doses of cortisol (Fig. 5), one group was injected with 1.5 mg/day of cortisol for three weeks, following which these animals, and another group which had been implanted three weeks previously, were unilaterally adrenalectomized. Various test doses of cortisol were then administered to both groups for 9 days, after which time the second adrenal was removed and weighed. The non-implanted animals continued to receive the daily 1.5 mg dose during the 9-day period, in addition to the test dose, to compensate for the continued presence of hypothalamic cortisol in the implanted group. Results are shown in Fig. 5, in which all points represent means.

Fig. 4.
Comparison of $\%$ adrenal weight difference in rats with intrahypothalamic cortisol implants and others treated systemically with daily injections of cortisol during the whole experiment.
obtained from 9 or more rats. In implanted animals, cortisol in doses of 1 mg or higher resulted in complete inhibition of CAH, a finding essentially similar to that previously noted in untreated unimplanted rats (see Table 2). At dose levels of 1, 2, and 4 mg per day, AWD's were very similar in systemically treated and unimplanted rats.

In order to determine whether the restoration of CAH in 21-day implanted rats could be prevented by increasing the exposure of forebrain regions to corticoids, double implants were placed in the median eminence and septum. Implants of dexamethasone in 24-gauge tubing were used, since these appear to be as potent as the large cortisol implants, although the smaller tubes are less damaging to the brain. Results are shown in Table 3. While both right adrenal weight and % AWD were normal in rats with bilateral cerebellar implants*, *** severe atrophy followed double implantation in the median

* In two previous studies from this laboratory, AWD's in untreated control rats under similar conditions were found to be respectively 62.7 ± 4.1% (Davidson & Feldman 1963) and 76.7 ± 6.6% (Feldman et al. 1966).

*** Right adrenal weight in 333 normal rats = 16.0 ± 0.2 mg.

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Table 3.
Effects of double implants of dexamethasone (in 24-gauge tubing) on right adrenal weight 21 days post-implantation, and on compensatory adrenal hypertrophy measured 9–10 days later.

<table>
<thead>
<tr>
<th>Implant locations</th>
<th>N</th>
<th>Right adrenal weight (mg)</th>
<th>Adrenal weight differences (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral cerebellum</td>
<td>6</td>
<td>17.4 ± 1.2</td>
<td>72.5 ± 6.6</td>
</tr>
<tr>
<td>Double median eminence</td>
<td>14</td>
<td>7.7 ± 1.0</td>
<td>36.8 ± 16.5</td>
</tr>
<tr>
<td>Bilateral septum</td>
<td>24</td>
<td>7.3 ± 0.2</td>
<td>21.9 ± 5.8</td>
</tr>
<tr>
<td>Septum and median eminence</td>
<td>9</td>
<td>8.4 ± 0.7</td>
<td>70.1 ± 17.8</td>
</tr>
</tbody>
</table>

eminence or septum, or simultaneous placement of one implant in each area. The AWD measure manifested great variability. It is evident, however, that the septum-median eminence combination resulted in a mean value essentially the same as in controls and that although double implants in other locations did reduce CAH somewhat, they did not abolish it entirely.

DISCUSSION

This study has revealed some interesting divergences between adrenal weight maintenance and two other types of pituitary-adrenal response in animals with chronic intrahypothalamic corticoid implants. The restoration of the adrenal ascorbic acid depletion response to acute surgical stress previously demonstrated in animals implanted for 21 days (Chowers et al. 1963), was shown here to be intact even at 70 days, although adrenals were severely atrophic. We have also observed, in unpublished experiments, significant ascorbic acid depletion 30, 42 and 50 days following hypothalamic implantation of cortisol. It was furthermore shown that the CAH response was present in rats with chronic cortisol implants resulting in adrenal atrophy. These results cannot be due to the persistence of irreversible atrophy, after total dissipation of the implanted steroid, since it is known that regrowth of the adrenal occurs promptly following cessation of long-term systemic administration of corticoids (Winter et al. 1950; Bodansky & Money 1954).

It is difficult to interpret the finding that adrenal ascorbic acid depletion following unilateral adrenalectomy was normal in chronically implanted rats with advanced adrenal atrophy. The plasma corticosterone response to stress (etherization and jugular venisection) is blocked in male rats implanted with cortisol, 15 days postoperatively (Davidson et al. 1965), and in females two months postoperatively (Jones & Levine, unpubl. observations). Conceivably, implanted rats may respond differently to different stresses, and discrepancies
have been reported between corticosterone secretion and ascorbic acid depletion (Slusher 1958; Staehelin et al. 1965). Interpretation of the ascorbic acid depletion in implanted rats is further complicated by the fact that initial ascorbic acid concentration was decreased and by possible changes in adrenal sensitivity to corticotrophin (ACTH).

Of particular interest is the finding that animals with severe and continuing adrenal atrophy may respond to the challenge of unilateral adrenalectomy with marked compensatory hypertrophy of the contralateral adrenal. The three-week period required for return of CAH corresponds with the period for adrenal ascorbic acid depletion noted in our earlier study (Chowers et al. 1963). Two alternative general interpretations for this phenomenon suggest themselves. The first would postulate a gradually decreasing release of cortisol from the implant. This might result in a partial inhibition of the «negative feedback controller» in the basal hypothalamus such that the secretion of adrenal weight-maintaining amounts of ACTH is prevented, although the stimulus of unilateral adrenalectomy could break through the partial block to result in CAH. In this interpretation are implicit two main assumptions, neither of which has been tested: a) a gradually decreasing dose of corticosteroid can result in a gradually increasing degree of adrenal atrophy and b) the feedback signal required to prevent adrenal weight maintenance is less powerful than that required to prevent CAH and ascorbic acid depletion in response to stress. Furthermore, this interpretation does not seem consistent with the finding that hypothalamic implantation of the more potent corticoid, dexamethasone, which produced a greater decrease in adrenal weight than did cortisol 21 days following implantation, did not inhibit CAH following unilateral adrenalectomy at this time.

The second possible interpretation of the dichotomy which develops between adrenal weight maintenance and compensatory hypertrophy in chronically implanted animals is suggested by the fact that this dichotomy was not seen following daily injection of cortisol. The difference between the two treatments is not merely a quantitative one due to the systemic treatment producing a greater overall adrenal inhibition, since adrenal weight in injected animals was higher at 14 days than in cortisol implanted rats, and at 21 days than in dexamethasone implanted animals. When only the basal hypothalamus is exposed to high regional concentrations of cortisol, a certain reorganization of the neural mechanisms underlying ACTH output might well occur in the weeks following corticoid implantation. Such an adaptation could result in recovery of certain adrenocorticotropic responses such as the response to unilateral adrenalectomy. However, in the case of systemic administration, when the whole organism is exposed to high corticoid concentrations, the postulated adaptation would not occur.

The finding (Davidson et al. 1967) that corticoid implantation in certain
extrahypothalamic regions of the forebrain results in pituitary-adrenal inhibition suggests that these areas, which are presumably unaffected by median eminence implants, might be related to the phenomenon under discussion. It was found (see Fig. 5) that systemic administration of cortisol prevented the CAH response in 21-day implanted animals and this could be interpreted as resulting from inhibition of the extra-median eminence mechanism which controls the ACTH release underlying CAH in chronically implanted animals. Attempts to inhibit such a possible extrahypothalamic mechanism by implantation of dexamethasone in the septum were not, however, successful. Clearly, the final interpretation of the differential recovery of certain pituitary-adrenal functions reported in this study to follow chronic hypothalamic implantation of corticoids must await further research.

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REFERENCES


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