The rise of plasma ACTH induced by ether is mediated through neural pathways entering the medial basal hypothalamus

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Abstract. The effect of ether stress on the release of immunoreactive ACTH was studied in rats with an antero-lateral cut around the medial basal hypothalamus. Ether failed to raise the plasma ACTH level of rats in which an antero-lateral hypothalamic cut and adrenalectomy had been performed 7 to 8 days previously. Plasma ACTH was also unchanged in rats exposed to ether 2 h after an antero-lateral cut. These data suggest that intact neural pathways entering the medial basal hypothalamus from the antero-lateral direction are necessary for the ACTH releasing action of ether stress.

It has been suggested (Halász et al. 1967) that the ‘hypophyseotrophic area’, a part of the medial basal hypothalamus is able to maintain the basal secretion of corticotrophin (ACTH) at a level above that in normal rats. Further studies reported that the secretion of corticotrophin releasing factor (CRF) of the isolated medial basal hypothalamus (MBH) can be increased by ether inhalation in adrenalectomized rats (Allen et al. 1974), and decreased by the feedback effect of glucocorticoid excess (Abe & Critchlow 1977). The idea that CRF producing cell bodies are located within the medial basal hypothalamus received support from recent findings demonstrating normal (Yasuda & Greer 1976) or slightly increased (Krieger et al. 1977) CRF content in the medial basal hypothalamus of rats with deafferentation of this hypothalamic region.

Recent evidence from this laboratory, however, failed to confirm the widely held hypothesis that the surgically isolated medial basal hypothalamus is capable of regulating to a large extent the activity of the pituitary-adrenal axis. We found that, in contrast to earlier claims (Halász et al. 1967; Palka et al. 1969), the surgically isolated medial basal hypothalamus does not maintain an increased pituitary-adrenal function under resting conditions (Stark et al. 1978). Other experiments showed that in rats with a carefully checked antero-lateral cut around the medial basal hypothalamus the stimuli of surgical trauma (Palkovits et al. 1976), electrical stimulation of the tuber cinereum (Makara et al. 1978), as well as ether inhalation (Makara et al., in press), failed to raise plasma corticosterone level, and more than 90% of the CRF activity in the well-defined stalk-median eminence area disappeared within one week of deafferentation (Makara et al. 1979).

These results suggested that CRF producing cell bodies are located outside the cut usually made in neuroendocrine experiments, and that their fibres enter the isolated area of the medial basal hypothalamus from an antero-lateral direction. Since this suggestion is apparently in conflict with the report showing that ether is capable of releasing ACTH in adrenalectomized rats with complete or antero-lateral deafferentation of the medial basal hypothalamus (Allen et al. 1974), experiments were designed to re-evaluate the effect of ether stress on plasma ACTH level in normal and in adrenalectomized rats with antero-lateral hypothalamic cut.
Materials and Methods

Male rats of CFY origin (bred in our Institute), weighing 200–300 g, were housed under standard conditions of lighting (12 h of light beginning at 06:00), humidity (55–75%) and temperature (24 ± 1°C). They were given pelleted rat food and tap water ad libitum.

Experiment 1

The rats were anaesthetized with pentobarbitone (Nembutal, Serva, 35 mg/kg ip), and sham operation or an antero-lateral cut was performed as described earlier (Makara et al. 1978) using a Halász-type knife (Halász et al. 1967) with 1.8 mm height and radius. Immediately after hypothalamic surgery all these rats were bilaterally adrenalectomized by dorsal approach and were given 1.5 mg of oxytetracyclin sc. After adrenalectomy the rats received 0.15 ml NaCl in the drinking water. These rats were stressed by ether inhalation 7 to 8 days later.

Experiment 2

Antero-lateral deafferentation was performed under ether anaesthesia, and the rats were exposed to ether inhalation 2 h later.

Ether stress consisted of placing the rats in an ether saturated jar for initiating anaesthesia which was maintained with a nose cone until decapitation after 3 min exposure. Controls were decapitated within 30 seconds of opening their home cage. Trunk blood was collected into chilled polystyrene tubes moistened with 7 mg ethylene diamine tetraacetate (EDTA) in 0.1 ml saline, and centrifuged immediately (1200 g; 15 min; 4°C). Plasma was stored at −20°C until measurement of ACTH using a radioimmunoassay (Rees et al. 1971) with an antibody raised against α-h1–32ACTH (Richter, Budapest) conjugated to bovine serum albumin. Alphaα1–39 ACTH (a gift from Dr. A. F. Parlow, NIH, Bethesda) was used for iodination and reference.

One ml plasma was extracted and assayed in duplicate as described (Rees et al. 1971), and the ACTH content of the unknown samples was calculated with help of standard dose-response curves obtained in 1 ml hypophysectomized rat plasma. Reproducible sensitivity of the assay was 20 pg/ml.

Using criteria described earlier (Palkovits et al. 1976) the configuration and completeness of the hypothalamic cuts was checked on serial coronal sections of 10 μm thickness taken at least at 50 μm intervals from the hypothalamic blocks embedded in paraffin. The sections were stained with crotonaldehyde-fuchsin (Brinkman & Bock 1970) for neurosecretory material, and counterstained with acid carmin.

The data are presented as arithmetic means and SEM, and have been statistically analysed by one-way analysis of variance.

Results

On histological examination the antero-lateral hypothalamic cuts around the medial basal hypothalamus began at the posterior edge of the optic chiasma and were about 1 mm from the midline at the coronal level of the anterior tip of the median eminence; they usually extended to at least the mid-median eminence level. Some rats were grouped separately, because they had an antero-lateral cut that left a gap at the base of the hypothalamus on one side starting in the lateral part of the retrochiasmatic area (lesion type 3 according to Palkovits et al. (1976)).

In experiment 1 basal plasma ACTH level was high 7 to 8 days after hypothalamic sham operation + adrenalectomy (basal ACTH level in normal rats (n = 7) was 57 ± 16 pg/ml) and showed a marked
The effect of ether inhalation (3 min) on plasma ACTH of rats 2 h after placing an antero-lateral cut (ALC) around the medial basal hypothalamus.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma ACTH (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>3 min ether</td>
</tr>
<tr>
<td>Sham operation</td>
<td>131 ± 42 (6)*</td>
<td>344 ± 55 (9)**</td>
</tr>
<tr>
<td>ALC</td>
<td>138 ± 33 (10)</td>
<td>152 ± 21 (13)</td>
</tr>
</tbody>
</table>

* Mean ± SEM (number of rats).
** Significantly different from all other groups ($P < 0.01$).

Further rise in response to ether inhalation ($P > 0.01$). In contrast, in adrenalectomized rats with an antero-lateral hypothalamic cut the basal ACTH level was significant lower ($P < 0.01$) and failed to change after ether inhalation ($P > 0.1$). The rats with a retrochiasmatic gap in the antero-lateral cut had low basal ACTH level but showed a significant ($P < 0.05$) ACTH release in response to ether inhalation (Fig. 1).

In experiment 2 rats with intact adrenals were subjected to ether stress 2 h after hypothalamic surgery, at a time when the CRF content of the stalk-median eminence region is not yet depressed (Makara et al., 1979). Even 2 h after hypothalamic sham operation ether induced a rise of plasma ACTH level, whereas in rats with an antero-lateral hypothalamic cut it proved to be ineffective (Table 1).

**Discussion**

The present results show that in adrenalectomized rats ether stimulated ACTH release is dependent on nerve fibres entering the medial basal hypothalamus from either anterior or lateral directions, consequently the site of action of ether on ACTH release seems to be outside the medial basal hypothalamus. These data are consistent with the hypothesis (Makara et al. 1979 and in press) that CRF containing perikarya send their axons from outside the medial basal hypothalamus towards the stalk and median eminence, so that a surgical cut severing such axons should prevent ether-induced ACTH secretion due to the lack of CRF release.

The relationship of CRF and vasopressin is not yet clear. Since the neuro-secretory fibres of the median eminence and neural lobe come from outside the medial basal hypothalamus, and a large proportion of them pass through the lateral part of the retrochiasmatic area, the present findings are not in contradiction with the suggestion that vasopressin fibres of the median eminence might be involved in the regulation of ACTH secretion (Zimmerman 1976); however, our present data do not provide direct evidence that vasopressin mediates the ether-induced ACTH release.

An alternative explanation is that ether activates afferent pathways to the medial basal hypothalamus that would convey excitation to CRF producing cells located in the vicinity of the median eminence. Nevertheless, this alternative possibility seems unlikely since CRF activity in acid extracts of the stalk and median eminence disappeared 7 to 8 days after cutting all antero-lateral afferents to the medial basal hypothalamus (Makara et al. 1979).

The possible ‘trophic’ dependence of CRF synthesis within the medial basal hypothalamus on afferents coming from outside the surgical cut has also to be considered, because the observations claiming ether-induced ACTH release even after complete hypothalamic ‘deafferentation’ are used not only as a strong argument in favour of an intrahypothalamic site of action of ether (Fieldman et al. 1970), but also as a proof for the existence of CRF producing perikarya within the surgically isolated hypothalamic tissue (Halász et al. 1967). If in our chronic experiments the synthesis of CRF and the responsiveness to ether diminished as a result of secondary trans-synaptic degeneration of CRF containing cell bodies within the medial basal hypothalamus, one would expect a normal response to ether shortly after (i.e. within a few hours) cutting the afferents, since secondary degeneration is generally considered to be a slow process, and since, within one day of hypothalamic surgery, the CRF content of the stalk-median eminence region was still unchanged (Makara et al. 1979). However, this explanation invoking a trans-synaptic trophic effect also seems unlikely, since ether failed to release ACTH in rats 2 h after placing antero-lateral cuts around the medial basal hypothalamus. The findings of Allen et al. (1974) could have been only partially confirmed, as we also found that the increased plasma ACTH level following adrenalectomy is largely dependent on antero-lateral neural input to the medial basal hypothalamus, but we failed to find an ether-induced elevation of plasma.
ACTH in adrenalectomized rats with completely transected antero-lateral nerve fibres. This discrepancy might be explained by small differences in experimental conditions: i.e. Allen et al. (1974) used a longer post-operative interval before measuring basal and ether-stimulated levels of ACTH. Nevertheless, we think the most important difference is that those studies were performed before the importance of fibres in the basal lateral part of the retrochiasmatic area was recognised in the mediation of stress-induced ACTH release (Palkovits et al. 1976), and so in their rats minor gaps of the hypothalamic cuts might have remained unnoticed. The ether-induced ACTH release we found in rats with gaps in the hypothalamic cut supports this interpretation.

Our data obtained with acute or chronic hypothalamic cuts cannot be easily reconciled with previous findings obtained in rats with forebrain removal (Matsuda et al. 1964; Dunn & Critchlow 1969), which indicate that 24 h after preparing a hypothalamic or a ‘median eminence’ island by removing most of the forebrain, the rats respond with a significant rise of plasma corticosterone to stressful stimuli under ether anaesthesia. One possible explanation for this apparent controversy is that the blood loss and stress associated with the severe surgery of forebrain removal might have sensitized either the acutely severed CRF containing nerve fibres, or the anterior pituitary, toward a further stress or to ‘tissue CRF’ (Brodish 1977). Those preparations might also have included more hypothalamic tissue than the medial basal hypothalamus.

Both previous findings (Allen et al. 1974) and the present findings show that the rise of plasma ACTH level that follows adrenalectomy is largely dependent on intact nerve fibres reaching the medial basal hypothalamus from an antero-lateral direction. These results clearly indicate that the decreased blood level of adrenocortical hormones stimulates ACTH secretion largely via the central nervous system, and has little, if any, effect on the anterior pituitary itself. Since there is convincing evidence that glucocorticoids inhibit ACTH release, partly via the hypothalamus (Jones 1978), and partly via an action on the anterior pituitary (for references see Yates & Maran 1974; Jones 1978), it seems possible that the site of action of corticoid excess is partly different from that of corticoid deficiency. Allen et al. (1974) interpreted their findings as suggesting that the site of glucocorticoid feed-back lies outside the hypothalamus, possibly in the amygdala (Allen & Allen 1975).

Since the CRF-producing cell bodies may be outside the surgically deafferented hypothalamic area we think that experiments using an antero-lateral hypothalamic cut cannot discriminate between the possibilities that decreased blood corticoid level act on CRF-producing cells, or that afferent pathways from outside the hypothalamus are necessary for increased ACTH release following adrenalectomy. The dissociation of the effects of adrenalectomy and ether in rats with gaps in the antero-lateral hypothalamic cut might be explained simply by suggesting that more intact CRF-containing fibres are necessary for a sustained elevation of ACTH secretion following adrenalectomy, than for the release of a short pulse of corticotrophin following the intense stimulus of ether inhalation.

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