Stimulation of the Hypothalamic–Pituitary–Adrenal Axis and Inhibition of Growth Hormone Release via Increased Central Noradrenaline Neuronal Activity by Urethane Anaesthesia in the Rat: Blockade by Clonidine

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Abstract

Computerized gas chromatography–mass spectrometry was used to measure precisely the hypothalamic levels of noradrenaline (NA), dopamine and serotonin together with those of their major neuronal metabolites 3,4-dihydroxyphenylethylene glycol (DHPG), 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid in normal male rats 45 min after stimulation of hypothalamic–pituitary–adrenal function by urethane (1–3 g/kg) administration. Urethane treatment resulted in a significant elevation of central noradrenergic neuronal activity (NNA) as assessed from marked rises in hypothalamic DHPG concentrations and the ratio (DHPG/NA). At the same time there was significant stimulation of ACTH and corticosterone release and inhibition of growth hormone release. These hormonal and central effects of urethane (but not anaesthesia) were inhibited when the α2-agonist clonidine (150 μg/kg) was co-administered. Urethane had no major effect on hypothalamic dopamine or serotonin status. We propose that the release of ACTH and the suppression of growth hormone release following urethane anaesthesia is a result of activation of central NNA and suggest that the hormonal responses are mediated via hypothalamic noradrenergic facilitation of corticotrophin releasing factor and somatostatin release to the anterior pituitary.

Introduction

Because urethane anaesthesia induces low circulating levels of growth hormone (GH) in the rat, it has been often used in neuroendocrine studies of rat GH secretion (Collu et al. 1972; Kato et al. 1973; Ruch et al. 1976; Martin et al. 1978). During urethane anaesthesia immunoreactive somatostatin release from the hypothalamus is markedly stimulated and this is thought to be responsible for the inhibition of GH secretion under these conditions (Martin et al. 1978; Chihara et al. 1979). Urethane administration also results in stimulation of the pituitary–adrenal axis in the rat (Hamstra et al. 1984). With respect to its hormonal effects, urethane resembles ether anaesthesia which also causes suppression of GH and stimulation of adrenocorticotropin (ACTH). Evidence has been presented to suggest that ether anaesthesia exerts its action on the hypothalamic–pituitary–adrenal axis via activation of central noradrenergic neurons (Smythe et al. 1983a, 1983b, 1983c; Johnston et al. 1985), facilitating release of corticotrophin releasing factor (CRF) and it seemed possible that urethane might stimulate ACTH release via similar pathways. The aim of the present study was to investigate this question by examining the effects of urethane administration on the neuronal activity of hypothalamic noradrenaline (NA) in the presence and absence of the α2-agonist clonidine. In order to provide a more complete picture of the effects of urethane on the major hypothalamic monoamines, the levels and metabolism of dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) were also examined so that any actions
exerted by urethane on the major monoamines might be then related to hypothalamic monoaminergic mechanisms responsible for somatostatin release from the hypothalamus. Brain levels of 3,4-dihydroxyphenylethylenglycol (DHPG) have been shown to be dependent on, and to provide an index of, the neuronal activity of NA (Warsh et al. 1981; Scatton 1982). Furthermore, our studies using gas chromatography-mass spectrometry (GC-MS) have indicated the hypothalamic ratio DHPG/NA to be an even better predictor of noradrenergic functional activity in the rat than DHPG alone (Smythe et al. 1983b) and this ratio was used in the present investigation as the main index of noradrenergic neuronal activity (NNA). Similarly, the neuronal activities of hypothalamic DA and 5-HT were assessed from the ratios 3,4-dihydroxyphenylacetic acid (DOPAC)/DA and 5-hydroxyindoleacetic acid (5-HIAA)/5-HT, respectively (Smythe et al. 1982, 1983a, 1983b).

**Materials and Methods**

**Animal Studies**

Outbred male rats of the Wistar strain were used throughout in these studies. The animals were fed *ad libitum* and subjected to a 12:12 light-dark cycle (lights on at 0700 h) at a temperature of 20–22°C. Twenty-four 60-day-old rats were divided into four groups of six and were injected i.p. with the following solutions: group 1 (controls), saline, 1 ml; group 2, urethane (ethyl carbamate, B.D.H. Chemicals, Poole, U.K.; 1·35 g/kg in 1 ml distilled water; group 3, clonidine hydrochloride (Catapres, Boehringer Ingelheim, Artarmon, N.S.W.) 150 μg/kg in 1 ml saline; group 4, a combination of clonidine (150 μg/kg) and urethane (1·3 g/kg) in 1 ml distilled water. The animals were killed by decapitation 45 min after injection at which time trunk blood was collected. The timing and dose of urethane was chosen to be compatible with previous studies (Collu et al. 1972; Kato et al. 1973; Hamstra et al. 1984). Brains were rapidly removed and the medial basal hypothalamus (MBH) was extracted as previously described (Smythe et al. 1982, 1983a, 1983b). The mean wet weight of the MBH samples for the studies reported here was 20·9 ± 1·5 (s.d.) mg.

**GC–MS Assay of Hypothalamic Tissue**

The MBH samples were mechanically homogenized in a 1 ml solution of 5 M formic acid in n-butanol (1:4 v/v) which contained calibrated amounts of the deuterated internal mass spectrometry standards d₃-NA, d₃-DHPG, d₃-DA, d₃-DOPAC, d₃-5-HIAA and d₄-5HT (Smythe et al. 1982, 1983a, 1983b). Computerized gas chromatography-mass spectrometry (GC–MS) was used to assay precisely and specifically MBH concentrations of NA and its primary neuronal metabolite DHPG. The GC–MS assays for NA, DHPG, DA, DOPAC, 5-HIAA and 5-HT were carried out using a Hewlett-Packard 5987A GC–MS data system with extraction and derivatization procedures identical to those previously described (Smythe et al. 1982, 1983a, 1983b). The between-assay coefficient of variation for the various compounds varied from 2·5 to 4·0%. Sensitivity was less than 200 fmol for each compound. All results are expressed as picomoles per milligram of tissue wet weight.

**Other Assays**

The corticosterone concentration in rat serum samples was assayed by a modification of the radioimmunoassay method of Carr et al. (1977). The precision of the assay as estimated by the intra-assay coefficient of variation was 5·1% at 92 nmol/l, 4·1% at 450 nmol/l and 4·5% at 920 nmol/l (n = 10, 12, 10 respectively). The interassay coefficients of variation were 12, 9·3, 11·9% (n = 11, 14, 11 respectively). The sensitivity was 5 nmol/l. Serum ACTH was estimated by radioimmunoassay using materials supplied by Immuno Nuclear Corporation (Stillwater, Missouri). The limit of sensitivity for the assay was 50 pg/ml and the intra- and interassay coefficients of variation were 15 and 30% (maximum) respectively. Serum rat GH (rGH) was assayed by radioimmunoassay using material supplied by the NIADDK, Bethesda, Maryland. Data are expressed in terms of rGH-RP-1. The interassay coefficient of variation was less than 9%. Serum rat insulin was assayed by radioimmunoassay using a rat insulin standard (Novo Research Institute, Bagsvaerd, Denmark). Intra- and interassay coefficients of variation were 6 and 7% respectively at 5 μU/l. Glucose was assayed using a Yellow Springs glucose analyser (YSI 32 AM, Yellow Springs, Ohio).
Statistics

Planned comparisons were made between data means after analysis of variance using Student's t-test.

Results

The effects of urethane administration on hypothalamic levels and metabolism of DA, NA and 5-HT and on serum levels of glucose, insulin, corticosterone, ACTH and rGH are shown in Table 1. Urethane caused marked and highly significant stimulation of hypothalamic DHPG and a concurrent suppression of NA levels which resulted in a more than doubling of the ratio DHPG/NA. These changes in the hypothalamus, along with the small increase in DOPAC, are essentially the same as those observed following ether anaesthesia in the rat (Smythe et al. 1983b). Marked stimulation of serum levels of glucose, corticosterone and ACTH also occurred in the urethane-treated group. At the same time serum rGH levels were totally suppressed (to 3% of controls). When clonidine was administered to the urethane-anaesthetized animals the effects of urethane on the hypothalamic DHPG/NA ratio, serum ACTH and rGH were essentially abolished (see Table 1).

Table 1. The effects 45 min after the administration of urethane (1·3 g/kg, i.p.) or a combination of urethane (1·3 g/kg) and clonidine (150 μg/kg, i.p.) on hypothalamic levels and metabolism of NA, DA and 5-HT and on serum levels of glucose, insulin, corticosterone, ACTH and GH in the rat

<table>
<thead>
<tr>
<th></th>
<th>Controls (saline)</th>
<th>Urethane</th>
<th>Clonidine</th>
<th>Urethane + clonidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPG (pmol/mg)</td>
<td>1·39 ± 0·06</td>
<td>2·23 ± 0·12***</td>
<td>1·25 ± 0·06**</td>
<td>1·27 ± 0·09**</td>
</tr>
<tr>
<td>NA (pmol/mg)</td>
<td>10·55 ± 0·30</td>
<td>8·14 ± 0·45***</td>
<td>10·70 ± 0·35**</td>
<td>9·19 ± 0·43*</td>
</tr>
<tr>
<td>Ratio DHPG/NA</td>
<td>0·132 ± 0·008</td>
<td>0·276 ± 0·14***</td>
<td>0·117 ± 0·006*</td>
<td>0·138 ± 0·018**</td>
</tr>
<tr>
<td>DOPAC (pmol/mg)</td>
<td>1·64 ± 0·11</td>
<td>2·02 ± 0·15*</td>
<td>1·22 ± 0·09*</td>
<td>1·40 ± 0·10**</td>
</tr>
<tr>
<td>DA (pmol/mg)</td>
<td>2·66 ± 0·17</td>
<td>2·94 ± 0·10**</td>
<td>2·60 ± 0·12**</td>
<td>2·30 ± 0·16**</td>
</tr>
<tr>
<td>Ratio DOPAC/DA</td>
<td>0·62 ± 0·05</td>
<td>0·70 ± 0·06**</td>
<td>0·47 ± 0·03**</td>
<td>0·61 ± 0·03**</td>
</tr>
<tr>
<td>5-HIAA (pmol/mg)</td>
<td>3·95 ± 0·18</td>
<td>3·57 ± 0·16**</td>
<td>3·70 ± 0·18**</td>
<td>3·75 ± 0·20**</td>
</tr>
<tr>
<td>5-HT (pmol/mg)</td>
<td>5·61 ± 0·19</td>
<td>5·51 ± 0·24**</td>
<td>6·45 ± 0·16**</td>
<td>6·24 ± 0·14**</td>
</tr>
<tr>
<td>Ratio 5-HIAA/5-HT</td>
<td>0·71 ± 0·02</td>
<td>0·65 ± 0·02**</td>
<td>0·57 ± 0·02**</td>
<td>0·59 ± 0·04**</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td>7·0 ± 0·19</td>
<td>9·83 ± 0·40***</td>
<td>14·6 ± 1·09***</td>
<td>14·14 ± 0·71***</td>
</tr>
<tr>
<td>Serum insulin (mU/l)</td>
<td>23·5 ± 2·9</td>
<td>26·5 ± 2·9**</td>
<td>12·5 ± 2·9*</td>
<td>17·3 ± 1·5*</td>
</tr>
<tr>
<td>Serum corticosterone (nmol/l)</td>
<td>110 ± 45</td>
<td>446 ± 40***</td>
<td>410 ± 60***</td>
<td>452 ± 80***</td>
</tr>
<tr>
<td>Serum ACTH (ng/l)</td>
<td>88·4 ± 8·4</td>
<td>150 ± 24***</td>
<td>79 ± 12**</td>
<td>80·6 ± 2·6**</td>
</tr>
<tr>
<td>Serum rGH (μg/l)</td>
<td>173 ± 103</td>
<td>5·6 ± 1·2**</td>
<td>410 ± 54*</td>
<td>203 ± 95**</td>
</tr>
</tbody>
</table>

Serum levels of glucose and corticosterone, however, remained elevated after clonidine treatment. This latter result was not unexpected and is consistent with the stimulation of glucose and corticosterone via a peripheral action of clonidine (Smythe et al. 1985a). Both groups of animals treated with clonidine exhibited significantly suppressed insulin levels (Table 1). Clonidine also exerted the effect of significantly increasing the hypothalamic levels of 5-HT and reducing the ratio 5-HIAA/5-HT which effect we have previously reported (Smythe et al. 1983a). As assessed by
their immobility, flaccid tone and non-responsivity to paw pressure, clonidine exerted no obvious effect on the degree of anaesthesia in the urethane-treated animals.

Discussion

A major effect of urethane anaesthesia on hypothalamic monoamine status in the rat was shown to be a large increase in NNA, contributed to by a concurrent rise in DHPG concentration and a decrease in NA concentration. This effect of urethane is similar to the action exerted on central NNA by ether anaesthesia in that the increase in NNA is associated both with an increase in hypothalamic DHPG concentrations and a fall in NA concentrations (Smythe et al. 1983b). Recently, Johnston et al. (1985), who used measurements of hypothalamic regional methoxyhydroxyphenylethylene glycol/NA ratios to assess NNA also found that ether anaesthesia increases central NNA.

The marked increase in hypothalamic NNA as a result of urethane treatment is consistent with our proposal that such a response might occur and thus facilitate CRF and ACTH release. In every model we have thus far examined in which central NNA is stimulated we have also observed concurrent increases in ACTH release (Smythe et al. 1983b, 1983c, 1984, 1985a, 1985b). More recently, we have proposed that liver glucose output is also mediated via central NNA (Smythe et al. 1984; Storlien et al. 1985) and we have shown that serum glucose concentrations in the rat are closely correlated with hypothalamic NNA (Smythe et al. 1984, 1985a, 1985b; Storlien et al. 1985). The measurement of glucose and the observation that its levels in serum are elevated after urethane in the present study adds further support for a facilitatory role of central NNA in the release of hepatic glucose. However, peripheral effects after clonidine administration in the rat result in marked stimulation of serum glucose and corticosterone levels (Smythe et al. 1985a) and this effect was observed in the present study, being typified by the concurrent inhibition of ACTH release due to the action of clonidine in the brain. The peripheral actions of clonidine effecting increased glucose levels appear, at least in part, to be mediated via $\alpha_2$-adrenoceptors inhibiting insulin release since $\alpha_2$-antagonists such as yohimbine have been shown to stimulate insulin release (Ahren et al. 1984). The present data is consistent with this proposal since significant suppression of insulin occurred in the clonidine-treated animals while urethane was without effect. It is not clear at which level in the pancreas clonidine exerts its action: it may be direct action on the $\beta$-cell or, conceivably, it may be mediated by $\alpha_2$-adrenergic stimulation of pancreatic somatostatin.

Another major hormonal response to urethane treatment in the rat is the inhibition of GH release (Collu et al. 1972; Martin et al. 1978). Since urethane has been shown to induce hypothalamic somatostatin release into pituitary portal blood (Chihara et al. 1979), it has been suggested that the inhibition of GH after urethane is specifically mediated via somatostatin (Martin et al. 1978). Moreover, evidence has been presented which suggests that noradrenergic afferents to the rat median eminence are involved in the augmentation of somatostatin release to the pituitary (Day and Willoughby 1980) and it was also recently proposed by Johnston et al. (1985) that the release of hypothalamic somatostatin is stimulated via NA pathways and that this is the mechanism for stress-induced GH suppression in the rat. The present data support these concepts. An alternative explanation for the
inhibition of GH is that it is due to suppression of GH releasing hormone (GHRH). Since our previous data indicate that rGH release via GHRH activity is facilitated via serotoninergic pathways (Smythe et al. 1983a, 1983b) we would expect that if inhibition of rGH after urethane was mediated via reduced GHRH activity, then 5-HT activity should be concurrently inhibited by urethane. However, no significant effect of urethane on 5-HT activity in the hypothalamus was observed in the present study and it thus seems that a direct inhibition of GH release occurs following urethane. We have noted also that the stress of central neuroglycopenia induced by 2-deoxy-D-glucose administration in the rat which is associated with marked stimulation of hypothalamic NNA (Smythe et al. 1984) also causes suppression of GH release without significantly affecting hypothalamic 5-HT activity (G. A. Smythe and R. M. Gleeson, unpublished observations).

That the effects of urethane treatment on ACTH and GH secretion in the present study were specifically related to stimulation of central NNA was further supported by the data obtained from the animals treated with the \( \alpha_2 \)-agonist clonidine. Clonidine acts centrally by stimulation of prejunctional \( \alpha_2 \)-autoreceptors and thus inhibits neuronal NA release (Aghajanian 1981; Chesselet 1984). Our previous studies and the relationship we have established between central neuronal NA activity and ACTH release (Smythe et al. 1983b, 1985a) are also consistent with clonidine acting as a central \( \alpha_2 \)-agonist at the dose used in the present investigation. If, on the other hand, it were acting as an \( \alpha_1 \)-agonist it would increase, rather than reduce, ACTH secretion. Additional evidence, consistent with clonidine exerting its centrally mediated effects on ACTH release via stimulation of \( \alpha_2 \)-adrenoceptors, is that the \( \alpha_2 \)-antagonist drug yohimbine has the opposite effects on ACTH release to those of clonidine (Smythe et al. 1983b). In the present studies clonidine completely reversed the urethane-induced stimulation of ACTH release and the inhibition of GH release. This result is consistent with the concept that in the presence of urethane anaesthesia these hormonal responses were being sustained by a central noradrenergic drive.

A significant rise occurred in hypothalamic DOPAC levels in the urethane-treated animals: increased DA metabolism without a change in the metabolic ratio is also a feature following ether stress (Smythe et al. 1983a). While the present data do not positively exclude a primary role for central dopaminergic pathways in the responses to urethane, it appears likely that the change in DA metabolism is secondary to altered NNA since it was blocked when clonidine was co-administered.

The present investigation has provided data which indicate that the ability of urethane anaesthesia to influence anterior pituitary hormone secretion may be due to a stimulation of central NNA. The data also provide support for the concept that, like CRF, somatostatin release from the hypothalamus is facilitated by a noradrenergic drive.

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References


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