L-Arginine is unlikely to exert neuroendocrine effects in humans via the generation of nitric oxide

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There is now considerable evidence that nitric oxide is an important neuroregulatory agent, but there has been very little investigation of its possible role in neuroendocrine mechanisms in humans. We have investigated the effects of two nitric oxide precursors, L-arginine and molsidomine, under basal conditions on the pituitary hormones growth hormone (GH), prolactin, luteinizing hormone, follicle-stimulating hormone, thyrotrophin, adrenocorticotrophin (ACTH) and vasopressin, and also on serum cortisol; we have also studied the effect of L-arginine on circulating prolactin, ACTH and cortisol in normal human subjects under hypoglycaemic stress. L-Arginine stimulated both GH and prolactin release under basal conditions but had no effect on the other hormones studied, while the nitric oxide donor molsidomine showed no effect on any hormone studied. L-Arginine potentiated the hypoglycaemia-stimulated release of ACTH but did not influence the rise in GH. The current studies suggest that the effects of L-arginine on the stimulation of GH and prolactin release are unlikely to be mediated via the generation of nitric oxide.

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Nitric oxide (NO) is known to play an essential role in the control of vascular tone (1), but recently has also been shown to act as a neurotransmitter both in the brain and the periphery (2). Nitric oxide is synthesized in the central nervous system by the enzyme NO synthase, a Ca2+-calmodulin-dependent enzyme that has recently been cloned and sequenced, utilizing L-arginine as substrate (3, 4). The released NO is thought to act principally by stimulating cytoplasmic guanylyl cyclase, resulting in the elevation of cGMP, although alternative second messengers may also be involved. Pharmacological NO donors include the drug molsidomine, whose metabolite sydnonimine-1 liberates NO. The presence of NO synthase has been demonstrated in several brain areas, particularly the hypothalamic paraventricular and supraoptic nuclei, as well as in the posterior pituitary (5). These hypothalamic nuclei are the major source of corticotrophin-releasing factor (CRH) and vasopressin. Nitric oxide has been shown to inhibit stimulated CRH and vasopressin release in rats (6, 7), but data in humans are few. Furthermore, while L-arginine is an established stimulus to hormone (GH) secretion in humans (8), it has not been established previously whether this effect is related to the generation of NO or not. We have therefore investigated the effects of L-arginine and molsidomine on a variety of neuroendocrine variables under basal conditions, and the effect of L-arginine during hypoglycaemic stress, in normal human subjects.

Subjects and methods

Subjects

Fourteen healthy adult male subjects (aged 21–35 years) were tested in two separate studies. In the first, eight subjects were investigated on four occasions in a randomized double-blind manner separated by at least 7 days: the limbs were placebo, L-arginine, molsidomine and the combination of L-arginine plus molsidomine. Following an overnight fast, an indwelling forearm cannula was inserted at 08.30 h and the first blood sample was taken at 09.15 h (−30 min). At 09.30 h (−15 min), 30 g/100 ml L-arginine in normal saline, 4 mg/100 ml molsidomine in normal saline, L-arginine plus molsidomine or an equivalent volume of saline as placebo was administered over 15 min. Blood was sampled at 15-min intervals for 3 h for assay of serum GH, prolactin, TSH, LH, FSH and cortisol, and for plasma ACTH and vasopressin.

In a second study, six subjects were investigated on two occasions in a randomized double-blind manner: the two limbs were insulin-induced hypoglycaemia alone and in combination with L-arginine. At 09.15 h
(−15 min), either 30 g of l-arginine in 100 ml of saline, or the saline alone, was infused for 15 min. At 09.30 h (time 0), an intravenous bolus of insulin (Actrapid, Novo; 0.15 U/kg) was administered. Blood was sampled for assay of blood glucose, serum GH, cortisol and plasma ACTH and vasopressin at 15-min intervals for 3 h.

In both studies, pulse and blood pressure were recorded with each blood sample. Plasma or serum was frozen and stored at −20°C until assay. Each subject gave written informed consent to the study.

Arginine was purchased from Pierrel (Milan, Italy), while the molsidomine (Corvaton) was a gift from Cassella (Frankfurt, Germany).

Assays

Serum GH (Nichols Institute, San Juan Capistrano, CA), prolactin (NETRIA, London, UK), TSH (Hycor Biomedical, CA), LH (Radim, Pomezia, Roma, Italy), FSH (Radim, Pomezia, Roma, Italy) and plasma ACTH (Nichols Institute, San Juan Capistrano, CA) levels were measured by immunoradiometric assay; serum cortisol (Immunotech International, Marseille, France) and vasopressin (Buhlmann Laboratories, Switzerland) levels were measured by radioimmunoassay. The intra-assay coefficients of variation of the assays were 4.2%, 6%, <4%, 4%, 5.6%, 5.1%, <5% and 5.3% for GH, prolactin, TSH, ACTH, LH, FSH, vasopressin and cortisol, respectively. Minimal detectable concentrations were 0.04 mU/l, 10 mU/l, 0.02 mU/l, 0.22 pmol/l, 0.2 U/l, 0.18 U/l, 0.6 pmol/l and 13 nmol/l, respectively. All samples were assayed in duplicate, and all samples taken from an individual subject were analysed in the same assay.

Statistical analysis

All hormonal data were subjected to analysis of variance for cross-over designs following calculation of the incremental areas under the curves computed using the trapezoidal method. Incremental area under the curve was taken as the area above the baseline at 0 min. Where data were not normally distributed, a non-parametric analysis of variance was used instead. Significance was taken as p < 0.05. Statistical analysis was carried out using SPSS for Windows 6.1 (SPSS Inc., Chicago, USA).

Results

In the first study, GH release was stimulated significantly by l-arginine. By contrast, molsidomine had no effect on its own, and in combination with l-arginine was not different to that of l-arginine alone (Fig. 1). Similarly, prolactin release was stimulated by l-arginine infusion, while again no effect of molsidomine on its own was observed and molsidomine had no significant effect on the l-arginine-stimulated release of prolactin (Fig. 2).

No significant change was observed with any of the treatments in vasopressin, ACTH, cortisol, LH, FSH or TSH levels (Table 1). Neither blood pressure nor pulse rate was significantly altered by any of the treatment regimens (data not shown).

In the second study, insulin produced a marked fall in blood glucose to <2.2 mmol/l in every subject, and this was not significantly different when the insulin was preceded by l-arginine (p = 0.3). The hypoglycaemia produced a rise in serum GH (Fig. 3) and vasopressin (data not shown) that was also not significantly altered by l-arginine. By contrast, the insulin-induced rise in plasma ACTH was enhanced significantly by preceding the insulin with an infusion of l-arginine (Fig. 4): l-arginine also appeared to potentiate the release of cortisol, although this did not quite attain statistical significance (p = 0.054).
Table 1. Incremental area under the curve for circulating hormone levels after L-arginine and molsidomine alone and in combination (mean ± SEM).^a |

<table>
<thead>
<tr>
<th></th>
<th>Vasopressin (pmol·l⁻¹·min⁻¹)</th>
<th>ACTH (pmol·l⁻¹·min⁻¹)</th>
<th>Cortisol (nmol·l⁻¹·min⁻¹)</th>
<th>FSH (U·l⁻¹·min⁻¹)</th>
<th>LH (U·l⁻¹·min⁻¹)</th>
<th>TSH (mU·l⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>−52.2 ± 20</td>
<td>−98.2 ± 101</td>
<td>−23715 ± 7697</td>
<td>5.6 ± 8.4</td>
<td>4.0 ± 29</td>
<td>−25 ± 3.0</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>−69.3 ± 29</td>
<td>−188 ± 174</td>
<td>−16908 ± 5161</td>
<td>9.3 ± 7</td>
<td>−10.8 ± 33</td>
<td>−20 ± 8.7</td>
</tr>
<tr>
<td>Molsidomine</td>
<td>−46.5 ± 52</td>
<td>−33.3 ± 34</td>
<td>−10739 ± 3801</td>
<td>−9.5 ± 9.5</td>
<td>64 ± 17</td>
<td>22 ± 21</td>
</tr>
<tr>
<td>L-Arginine + molsidomine</td>
<td>−31 ± 16</td>
<td>117 ± 121</td>
<td>−3957 ± 6825</td>
<td>0.76 ± 20.7</td>
<td>56 ± 26</td>
<td>0.4 ± 19</td>
</tr>
</tbody>
</table>

^a None of the drug data were significantly different from those after the placebo.

Discussion

For many years, intravenous L-arginine administration has been used as a reliable test of GH secretion (8), and various recent studies have sought to elucidate the mode of action of L-arginine in promoting GH release. It has generally been suggested that L-arginine inhibits somatostatin secretion, although this is not universally agreed (9, 10). However, other neurotransmitter intermediates in this stimulatory pathway have not previously been investigated in depth.

In the rat, it is well established that the generation of NO may induce marked neuroendocrine changes, and in particular NO has been shown to inhibit the release of vasopressin both in vitro and in vivo (6, 7). Both excitatory and inhibitory effects of NO generation have been shown on CRH release in vitro, but recent in vivo data again suggest a predominant inhibitory modulation (11). With regard to the gonadotrophins LH and FSH, most studies have demonstrated that NO will stimulate the release of hypophysal GnRH (12–14). Studies on GH and prolactin have been fewer, but it has been suggested that NO may inhibit the release of GH by a direct pituitary effect (15), but may stimulate prolactin via the hypothalamus (16). In spite of these investigations, data supporting any putative role for NO in the modulation of hypothalamic hormone release in humans have been scanty. In most animal models, increased NO generation has been demonstrated when the substrate for nitric oxide synthesis, L-arginine, is added, and the effect of such L-arginine has generally been similar to that of other NO donors, such as molsidomine, nitroprusside and related drugs. We therefore speculated that the marked stimulation of prolactin and GH release in the human may be mediated via the generation of endogenous NO, and sought to prove this by the parallel use of another NO donor, molsidomine, and also by looking at the effect of L-arginine on ACTH and vasopressin, both of which are known to be inhibited by NO in the rat. However, contrary to expectation, no evidence was found to justify this speculation. In the first instance, molsidomine alone had no effect on any hormone tested, although it was used at a dose that is known to be pharmacologically effective in the periphery in the human (17). L-arginine was also shown in the present study to have no effect on the basal or hypoglycaemia-stimulated release of vasopressin. However, in a recent human study the hypoglycaemia-induced release of vasopressin was shown to be augmented by the NO synthase inhibitor N, G-nitro-L-arginine methyl ester (L-NAME) (18), suggesting that the effects of administered L-arginine may not after all act through the NO pathway within the central nervous system. In a

Fig. 3. Arginine did not change the elevation of serum GH (mean ± SEM) after the administration of insulin. ITT = insulin tolerance test.

Fig. 4. Arginine enhanced the effect of hypoglycaemia on plasma ACTH (mean ± SEM). ITT = insulin tolerance test.
study on patients with migraine, l-arginine was unable to trigger headache while causing changes in cerebral blood flow, whereas other NO donors were shown to induce pain attacks: this again shows separation of the effect of l-arginine and that of NO donors (19). A recent study also suggests that the effect of intravenous arginine on hormonal changes in humans is equal for both the l- and d-stereoisomers, implying that the NO pathway may not be involved in these processes because the NO synthase only binds the l-isomer (20). Our studies indicated that l-arginine potentiated the hypoglycaemia-stimulated release of ACTH, although it had no effect under basal conditions. We did not record any clear blood pressure changes, but l-arginine might still have produced minor haemodynamic effects which could have stimulated the hypothalamo-pituitary-adrenal axis to cause ACTH release. Our data are also consistent with the view that the stimulation of GH and prolactin release in the human is not mediated via a pituitary effect of NO. However, the site of stimulation of GH and prolactin release by l-arginine, whatever its intermediary neurotransmitter, could still be through a central neural action located within the blood/brain barrier, because molsidomine penetrates this poorly (U. Schindler, pers. comm.). The pituitary and median eminence are outside the blood/brain barrier, and the lack of any molsidomine effect indicates that there is unlikely to be any interaction of l-arginine as an NO donor at these sites. In addition, it is at least possible that NO is not an inhibitory modulator of the pituitary-adrenal axis or vasopressin release in humans. However, while a combination of such arguments could be used to justify speculation that l-arginine acts as an NO donor within the neuroendocrine axis in humans, the current data offer no direct support for this hypothesis. Furthermore, neither l-arginine nor molsidomine was able to stimulate gonadotrophin release, which also might have been expected from the rat model. In a recent abstract, it was found that the NO donor nitroglycerin could inhibit the GnRH-stimulated release of LH, while having no effect on basal LH or FSH release (21). This is in accordance with our findings on basal levels. However, the reported inhibition of GnRH-stimulated LH release would implicate a direct pituitary effect of NO, which we believe the present study has rendered as an unlikely locus for the effect of l-arginine on GH and prolactin release.

In summary, l-arginine stimulated both GH and prolactin release under basal conditions and potentiated the hypoglycaemia-stimulated release of ACTH, while the NO donor molsidomine showed no effect on any basal hormone level studied. The current studies suggest that the effects of l-arginine on the stimulation of GH and prolactin release are unlikely to be mediated via the generation of NO, but further studies with NO donors that penetrate the blood/brain barrier more effectively would be of interest.

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References


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