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Growth hormone release evoked by electrical stimulation of the arcuate nucleus in anesthetized male rats

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Plasma growth hormone (GH) concentrations were measured following electrical stimulation of either the arcuate nucleus or the median eminence in urethane-anesthetized male rats. While electrical stimulation of the arcuate nucleus elicited a large pulse of GH secretion, stimulation of the median eminence was relatively ineffective. For stimulation of the arcuate nucleus, the frequency dependence of stimulus–secretion coupling for GH release was investigated by delivering 3 differing patterns of electrical stimulation, each of 2 min duration and containing 1,200 stimulus pulses: 10 Hz continuous; 20 Hz (10 s on/10 s off); and 50 Hz (2 s on/8 s off). To examine the effect of increasing the duration of the 50 Hz stimulus train on evoked GH release, a further three stimulation protocols were also tested: 50 Hz (2 s on/8 s off); 50 Hz (3 s on/7 s off) and 50 Hz (4 s on/6 s off). While evoked GH release (per stimulus pulse) was not significantly different for various frequencies of stimulation, it was greatly potentiated by increasing the duration of 50 Hz stimulus trains. These findings suggest that GH release is not linearly related to the activation of GRF neurons but is strongly facilitated with increases in burst duration.

INTRODUCTION

In conscious, but not anesthetized, adult male rats growth hormone (GH) is released from the anterior pituitary gland in a pulsatile manner, with peaks of > 200 ng/ml plasma every 3.3 h[5]. Passive immunization studies indicate that the hypothalamic peptides GH-releasing factor (GRF) and somatostatin are responsible for controlling different aspects of the GH secretory pattern: while release of GRF into portal blood is necessary for GH release to occur[29], somatostatin controls both the GH pulse frequency and interpulse GH concentrations, by inhibiting GH secretion[27]. The effects of GRF and somatostatin on GH release are highly dependent on their pattern of exposure, but at present it is not known how the electrophysiology of these two neuronal systems relate to pulsatile GH secretion.

For peptide-secreting neurons, most information is known about the hypothalamic magnocellular neurons releasing oxytocin and vasopressin. In this system, the amount of hormone released from the nerve terminal is critically dependent on the pattern of neuronal activity recorded at the cell body[24,28]. Using an in vitro preparation of rat neural lobe, Bicknell et al.[2] compared the amounts of oxytocin and vasopressin released for a constant number of stimulus pulses at various frequencies of stimulation; the optimal pattern for oxytocin release (50 Hz for 3 s) was very different to the optimal pattern for vasopressin release (13 Hz for 12 s). Indeed the patterns of electrical activity generated at the cell bodies of oxytocin and vasopressin neurons, when activated, are almost identical to the stimulation patterns most effective for hormone release[1].

In the present study, an in vivo stimulus–secretion coupling approach was used to investigate whether it is possible to elicit a pulse of GH secretion by electrical stimulation of the arcuate nucleus (the site of the GRF cell bodies), and if so, to investigate the optimal parameters for eliciting GH release. A preliminary account of the data has been reported previously[8].

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MATERIALS AND METHODS

Surgical procedures

Adult male Wistar rats, from our own colony, were used at 250–450 g b.wt. All experiments were performed under urethane (ethyl carbamate) anesthesia (1.25 g/kg b.wt., i.p.). A silastic cannula filled with heparinised isotonic saline was inserted into the jugular vein for blood sampling and for infusing human GRF (hGRF (1–44), Peninsula Laboratories, CA, USA). A double lumen cannula was used for experiments requiring an i.v. infusion of rat GRF (rGRF (1–43), Bachem, CA, USA). Stimulating electrodes (Clark Electromedical, Reading, UK) were positioned using stereotaxic coordinates[23], and their positions verified later by standard histological techniques[30]. For stimulation of the median eminence, a side-by-side stainless-steel electrode (SNEX-200X) was lowered from the dorsal surface of the brain at an angle of 3°, such that both of the electrode tips were on the mid-line of the median eminence (2.8 mm caudal to bregma, on mid-line, 10 mm deep). The arcuate nucleus was stimulated bilaterally (2.8 mm caudal to bregma, 0.2 mm on either side of the mid-line, 9.9 mm deep), using two concentric bipolar stainless-steel stimulating electrodes (SNEX-100X). The electrodes were connected to a gated pulse generator which created matched biphasic pulses (amplitude 1.0 mA, duration 1.0 ms). The experimental procedure commenced 1 h after completion of the surgery.

Blood sampling and hormone measurement

For each experiment, blood samples (0.4 ml) were removed before, 5 min and 10 min after the end of each electrical stimulation/GRF injection. Plasma was separated from whole blood by centrifugation. The red cells were resuspended in saline and returned to the rat. Plasma was assayed for either GH alone, or for both GH and luteinising hormone (LH). Radioimmunoassay of rat GH was carried out using 30 μl plasma diluted in a final volume of 300 μl (see ref. 6 for assay details). In different assays the detection limit varied from 0.33 to 0.83 ng/ml. Radioimmunoassay of rat LH was carried out using 20 μl plasma diluted in a final volume of 250 μl (modified from ref. 3). All LH measurements were made in a single assay and the detection limit was 0.03 ng/ml plasma. Reagents for both assays were supplied by NIDDK.

Electrical stimulation of either the arcuate nucleus or the median eminence using a high frequency intermittent stimulus

Stimulation at 50 Hz (5 s on/7 s off, 5 min) was applied either to the median eminence ($n = 10$) or to the arcuate nucleus ($n = 6$). 1 h after the stimulation each rat was given a bolus i.v. injection of 2 μg hGRF, which elicits a maximal release of GH to ensure that the pituitary was responsive to GRF.

Electrical stimulation of the arcuate nucleus using 3 different frequencies of stimulation

Each rat ($n = 12$) was stimulated 3 times using 3 different protocols for electrical stimulation and the order of presentation was varied according to a balanced design which controlled for order effects. The number of stimulus pulses was constant (1,200) for each protocol, but the frequency varied as follows: Protocol A (10 Hz continuous), B (20 Hz, 10 s on/10 s off) and C (50 Hz, 2 s on/8 s off). Each stimulus was of 2 min duration. 1 h after the last stimulus, each rat was given a bolus i.v. injection of 2 μg hGRF.

Electrical stimulation of the arcuate nucleus using a 50 Hz stimulus of 3 different stimulus train durations

Each rat ($n = 12$) was stimulated 3 times using 3 different protocols for electrical stimulation and the order of presentation was varied according to a balanced design. The protocols for stimulation were: C (50 Hz, 2 s on/8 s off), D (50 Hz, 3 s on/7 s off) and E (50 Hz, 4 s on/6 s off). Each stimulus was of 2 min duration. 1 h after the last stimulus, each rat was given a bolus i.v. injection of 2 μg hGRF.

Intravenous infusions of rGRF

Four different doses of rGRF were administered to each rat ($n = 24$) at 1 h intervals. The order of presentation was varied according to a balanced design, and doses of either 2 or 8 μg rGRF were administered last. A total of 7 different doses of rGRF were tested, ranging from 0.094 μg to 8 μg, and these were infused over a 2 min period in isotonic saline. The number of times each dose was tested is indicated in Fig. 5.

RESULTS

Plasma GH concentrations were measured following electrical stimulation of either the arcuate nucleus or the median eminence (Fig. 1). The arcuate nucleus was stimulated with repeated 50 Hz stimulus trains (5 s on/7 s off; 5 min; $n = 6$). Basal GH concentration varied from 0.2 to 1.6 ng/ml plasma between rats (mean ± S.E.M. = 0.6 ± 0.1 ng/ml). Electrical stimulation evoked a large increase in plasma GH concentration (mean ± S.E.M. = 61 ± 28-fold at 5 min; 72 ± 27-fold at 10 min; $P < 0.05$ at 5 min and 10 min, Signed Rank test, compared to control values). The same stimulation protocol, when applied to the median eminence ($n = 10$) was less effective for eliciting GH secretion. Basal GH concentrations, before median eminence stimulation, varied from 0.2 to 1.3 ng/ml plasma between rats (mean ± S.E.M. = 0.7 ± 0.1 ng/ml). The stimulus evoked a significant increase in plasma GH concentration (mean ± S.E.M. = 4.2 ± 2.2-fold at 5 min; 4.7 ± 3.4-fold at 10 min; $P < 0.01$ at 5 min and 10 min, Signed Rank Test, compared to control values) but the increase in plasma GH concentration was significantly smaller for median eminence stimulation than for arcuate nucleus stimulation ($P < 0.01$ at 5 min, $P < 0.001$ at 10 min, Mann–Whitney U-test).

At the end of each experiment a bolus i.v. injection of 2 μg hGRF elicited a marked increase in plasma GH concentration. In different experiments GH concentrations before GRF injection varied from 0.9 to 1.7 ng/ml plasma (mean ± S.E.M. = 1.2 ± 0.2 ng/ml). The increase in plasma GH concentrations varied from 32 to 81-fold at 5 min after injection (mean ± S.E.M. = 57 ± 14-fold) and from 58 to 73-fold at 10 min after injection (mean ± S.E.M. = 66 ± 4.2-fold; $P < 0.001$ for all experiments, Signed Rank test, compared to pre-injection values). The concentrations of GH obtained were comparable to the endogenous GH pulses observed in conscious male rats[5].

To investigate the frequency dependence of stimulus–secretion coupling for GH release, three differing protocols for stimulation were used: A (10 Hz continuous), B (20 Hz, 10 s on/10 s off) and C (50 Hz, 2 s on/8 s off). Mean basal GH concentrations varied from 0.3 ng/ml to 1.2 ng/ml plasma (mean ± S.E.M. = 0.6 ± 0.05 ng/ml). Each stimulation protocol

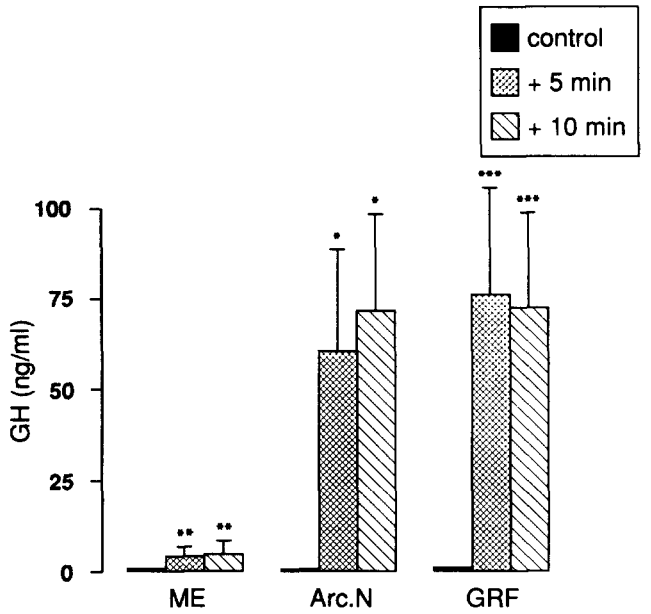

Fig. 1. Plasma GH concentration following electrical stimulation of either the arcuate nucleus (Arc.N, $n = 6$) or the median eminence (ME, $n = 10$) in urethane-anesthetized male rats using the stimulation protocol 50 Hz (5 s on/7 s off) for 5 min. A bolus i.v. injection of 2 μg hGRF was administered 1 h after the last stimulus. Blood samples were removed before, 5 min and 10 min following each stimulation/hGRF injection. The data are expressed as mean plasma GH concentration ± S.E.M. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Signed Rank test, compared to control values).

elicited a significant increase in plasma GH concentration (Fig. 2). The increase in plasma GH measured in samples at 5 min following stimulation for protocols A, B and C, were 14 ± 6.9-fold, 8.6 ± 6.7-fold and 11 ± 3.7-fold, respectively. The mean GH concentrations measured at 5 min and 10 min following each stimulation did not differ significantly for the 3 protocols (paired t-test, Fig. 2).

The optimal stimulus parameters for GH secretion were further investigated using the stimulation protocols: C (2 s on/8 s off), D (3 s on/7 s off) and E (4 s on/6 s off). Mean basal GH concentrations varied from 0.3 to 3.2 ng/ml plasma between rats (mean ± S.E.M. = 1.0 ± 0.3 ng/ml). Each protocol for stimulation elicited a significant increase in plasma GH concentration (Fig. 3), but the amount of GH released was very different for the 3 protocols. The increase in plasma GH measured in samples at 5 min following stimulation with protocols C, D and E, were 6.9 ± 1.5-fold, 43 ± 15-fold and 64 ± 15-fold, respectively. Protocol E contained twice the number of stimulus pulses as protocol C, yet the percentage increase in GH release was much more than double. Hormone release was calculated as release per stimulus pulse for each protocol, after first normalising the data to total release measured in each experiment, to minimise the inter-animal variation and effects due to variability in stimulating electrode position (Fig. 4). From this analysis the mean GH release per stimulus pulse was 2.6- and 4.8-fold larger for 3 s and 4 s stimulus trains than for 2 s stimulus trains. From these data, it may be inferred that for a 4 s train of stimulation at 50 Hz, 12% of the hormone release is in response to the first 2 s of the train, 31% during the 3rd second and 57% in the 4th second.

The amount of GH released with increasing doses of rGRF was measured to investigate the possibility that this facilitation of GH release with longer burst duration was due to non-linearity in the GH-releasing potential of GRF at the level of the pituitary. To make comparisons between GH release following a 2 min period of electrical stimulation and rGRF-induced release, various doses of rGRF were infused intravenously over a 2 min period. GH release was not observed at doses of rGRF below 0.184 μg and was comparable to stimulus-induced release at very high doses of rGRF (Fig. 5). The sum of the absolute values for GH release at 5 min and 10 min following stimulation (basal subtracted) for a 2 min infusion of 0.36 μg rGRF were of a similar magnitude to that elicited by protocol C (50 Hz, 2 s on/8 s off, 2 min). However, a

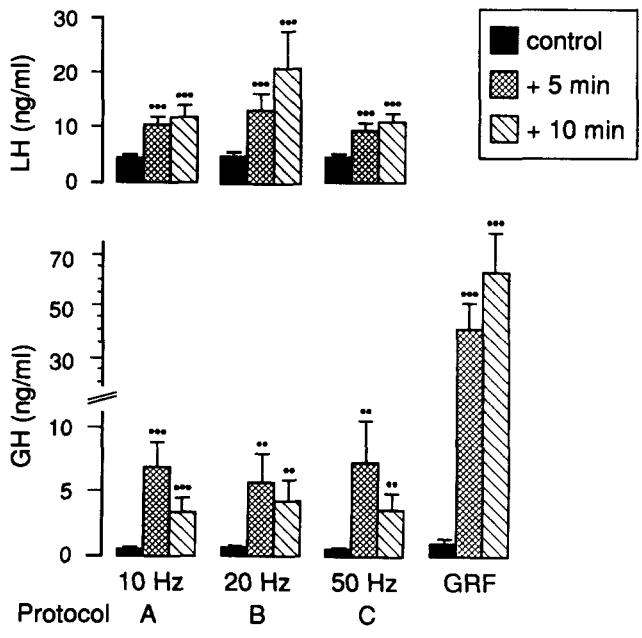

Fig. 2. Plasma GH and LH concentration following electrical stimulation of the arcuate nucleus in urethane-anesthetized male rats using different frequencies of stimulation. Each rat ($n = 12$) was stimulated using the protocols A (10 Hz, continuous), B (20 Hz, 10 s on/10 s off) and C (50 Hz, 2 s on/8 s off), and a bolus i.v. injection of 2 μg hGRF was administered 1 h after the last stimulus. Blood samples were removed before, 5 min and 10 min following each stimulation/hGRF injection. The data are expressed as mean plasma GH/LH concentration ± S.E.M. (** $P < 0.01$, *** $P < 0.001$, Signed Rank test, compared to control values).

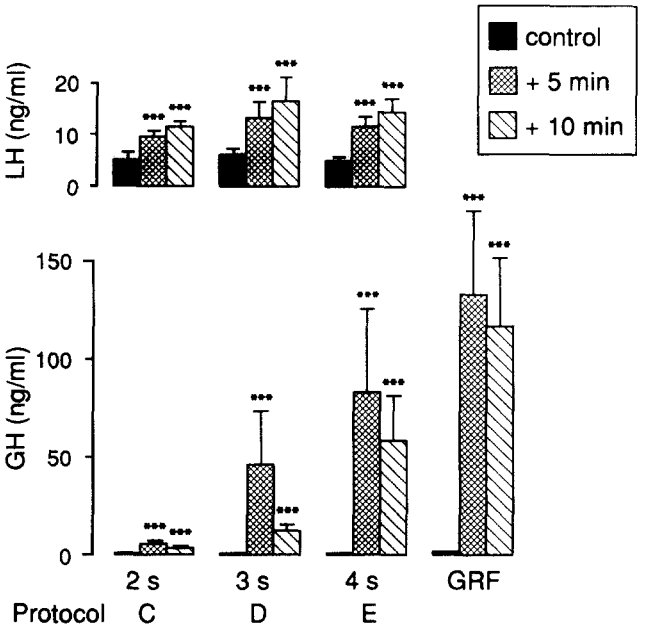

Fig. 3. Plasma GH and LH concentration following electrical stimulation of the arcuate nucleus ($n = 12$) in urethane-anesthetized male rats using 50 Hz stimulations of increasing stimulus train durations. Each rat was stimulated using the protocols: C (50 Hz, 2 s on/8 s off), D (50 Hz, 3 s on/7 s off) and E (4 s on/6 s off) and a bolus i.v. injection of 2 μg hGRF was administered 1 h after the last stimulus. Blood samples were removed before, 5 min and 10 min following each stimulation/hGRF injection. The data are expressed as mean plasma GH/LH concentration $\pm$ S.E.M. ($P < 0.001$, Signed Rank test, compared to control values).

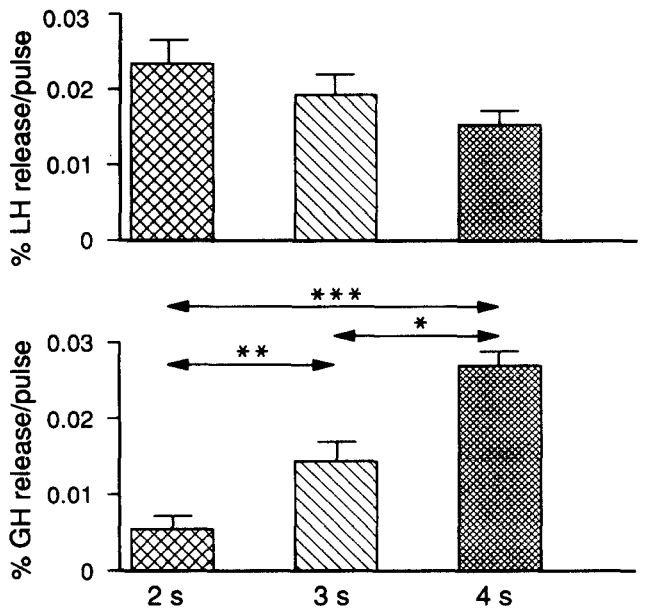

Fig. 4. Plasma GH and LH concentration following electrical stimulation of the arcuate nucleus in urethane-anesthetized male rats ($n = 12$) using 50 Hz stimulations of increasing stimulus train durations. The total amount of hormone released as a result of each stimulation (at 5 and 10 min, basal subtracted) is normalised to total release for each rat, and expressed per pulse of stimulation (mean $\pm$ S.E.M.; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Signed Rank test).

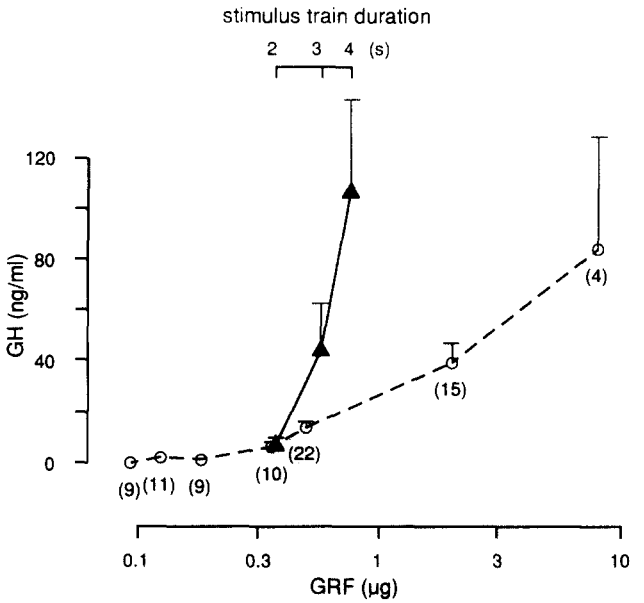

Fig. 5. A comparison between the amount of GH released by increasing doses of rGRF with that elicited by increasing the duration of the stimulus train applied to the arcuate nucleus in urethane-anesthetized male rats. The The plasma GH responses following various doses of hGRF administered over a 2 min period (○) were measured at 5 min and 10 min following each infusion. The numbers in brackets indicate the number of times each rGRF dose was tested. This was compared to GH concentrations following electrical stimulation of the arcuate nucleus with 50 Hz stimulations of increasing stimulus train duration (▲). Data are expressed as the mean ($\pm$S.E.M.) sum of GH concentrations at 5 min and 10 min following rGRF infusion/electrical stimulation (basal subtracted).

doubling of the number of stimulus pulses (4 s on, rather than 2 s on) was much more effective in releasing GH than a doubling of the dose of rGRF (Fig. 5). Hence, the large facilitation of GH secretion by increasing the 50 Hz stimulus burst duration from 2 to 4 s reflects a non-linearity in hypothalamic releasing factor secretion and does not reflect a non-linear GH response to GRF at the level of the pituitary.

Basal LH concentrations varied from 0.1 to 0.8 ng/ml plasma between rats (mean $\pm$ S.E.M. = 0.3 ± 0.1 ng/ml) (Figs. 2 and 3). All of the stimulation protocols tested (A–E) elicited a small but significant increase in plasma LH. By contrast to GH, LH release was not dependent on either the frequency or duration of stimulation for the 2 groups of stimulation protocols tested.

DISCUSSION

To our knowledge, this is the first report that brief electrical stimulation of the arcuate nucleus of urethane-anesthetized male rats can elicit a pulse of GH secretion which is comparable in magnitude to the spontaneous GH pulse observed in conscious male rats. Previous attempts to elicit GH secretion in urethane-anesthetized rats by electrical stimulation of dis-

crete regions of the hypothalamus[16] or median eminence[10] have been unsuccessful, although in pentobarbitone-anesthestized rats, a significant increase in plasma GH concentration has been demonstrated following stimulation either in the region of the ventromedial nucleus/arcuate nucleus[15] or the ventromedial nucleus alone[13].

In contrast to the large GH response to stimulation of the arcuate nucleus, we observed only a small GH release in response to median eminence stimulation. The relative ineffectiveness of median eminence stimulation for eliciting GH secretion is probably attributable to the co-activation of somatostatin neurosecretory terminals in the median eminence, since somatostatin is a highly potent inhibitor of GH secretion at the level of the pituitary gland[11]; and since electrical stimulation of the median eminence (using similar stimulation parameters to those used in the present study) has previously been shown to elicit a large increase in somatostatin concentration in portal blood[19]. Stimulation of the arcuate nucleus appears not to have resulted in direct activation of the nerve terminals in the median eminence since evoked LH release, reflecting LH-releasing hormone secretion from terminals in the median eminence, was low in the present experiments, and we would accordingly attribute the efficacy of arcuate stimulation in evoking GH release to successfully minimising evoked secretion of somatostatin.

Evoked GH release did not appear to be frequency-dependent, for the range 10–50 Hz, in the manner of oxytocin or vasopressin release from the neural lobe[1], since, in a direct comparison, the same number of stimulus pulses given at different frequencies of stimulation were equally effective in eliciting GH release. However, GH release was greatly potentiated by increasing the duration of the 50 Hz stimulus train from 2 to 4 s. This potentiation was very much larger than could be accounted for by the deviation from linearity between the amount of GH released and differing amounts of exogenously infused rGRF (Fig. 5). The potentiation appears therefore to result either from a non-linear facilitation of GRF release with increasing stimulus duration, or from a progressive reduction in somatostatin secretion with increasing stimulus duration. Given our inference that evoked somatostatin secretion is minimal with arcuate stimulation, the former interpretation is more likely.

Unlike Bicknell et al. who measured oxytocin and vasopressin release directly from the terminals[2], we must be cautious as our inference of 'GRF' release is indirect. The optimal pattern of GRF neuron activation for pulsatile GH secretion would probably involve a burst of high frequency discharge, since 3 or 4 s bursts of high frequency arcuate stimulation were significantly more effective for eliciting a pulse of GH secretion than a continuous low frequency (10 Hz) stimulus. However the optimal burst length appears to be greater than the burst duration of high frequency discharge recorded at oxytocin cell bodies during the milk ejection reflex since oxytocin neurons fire at a high frequency but only for short bursts of 1–2 s[24,28]. Nevertheless, the duration dependence remains and may suggest that the GRF discharge must be of longer duration than a typical 'oxytocin' milk ejection burst.

GRF neurons are distributed throughout the arcuate nucleus[12]. The experimental design used, with stimulating electrodes positioned bilaterally in the arcuate nucleus, is such that all GRF neurons are unlikely to be excited by the stimulus. Previous authors have demonstrated that stimulus pulses of magnitude similar to those used in the present study do not spread far within brain tissue[9]. Indeed, as already mentioned, plasma LH concentrations were relatively weakly affected for all stimulation protocols tested, indicating that current spread from the arcuate nucleus to the median eminence probably does not contribute substantially to hormone release. Thus, the approximation that 12 bursts of 4 s duration in a 2 min period are required to elicit a GH-secretory response, equivalent to spontaneous GH pulses, is an over-estimate of the maximum activation required.

Spontaneous pulsatile GH secretion is blocked under urethane anesthesia[4]. Since electrical stimulation of the arcuate nucleus elicits a pulse of GH secretion in urethane-anesthetized rats, this would suggest that urethane blocks GH secretion not by an inhibitory action either at the pituitary or the GRF nerve terminals, or simply as a result of enhanced somatostatin release into portal blood, but by suppressing the excitability of GRF cell bodies, whether directly or indirectly by suppressing the activity of excitatory afferents. Electrophysiological recordings from unidentified 'tuberoinfundibular' neurons have demonstrated that a large proportion of these cells are silent under urethane anesthesia and very few fire above 5 Hz[14,25,26].

It is possible that stimulation of the arcuate nucleus elicits the release of neuroactive substances other than GRF to influence GH secretion. There is accumulating evidence for the co-existence of dopamine with GRF[17,21] and dopamine may potentiate GH release by inhibiting somatostatin release from nerve terminals[17]. Several neuropeptides may also be co-released with GRF, such as neuropeptide Y[7] and galanin[18]. Galanin stimulates GH release when administered by either i.v. or i.c.v. injection[20,22], and this may reflect a modulatory

action either at the GRF cell body or nerve terminal[20]. However, since these putative GH secretagogues are all co-localized with GRF our inferences about the likely discharge characteristics of GRF neurones in eliciting pulsatile GH secretion are not significantly influenced by these considerations.

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REFERENCES

1 Bicknell, R.J., Optimizing release from peptide hormone secretory nerve terminals, *J. Exp. Biol.* 139 (1988) 51–65.
2 Bicknell, R.J., Brown, D., Chapman, C., Hancock, P.D. and Leng, G., Reversible fatigue of stimulus–secretion coupling in the rat neurohypophysis, *J. Physiol.*, 348 (1984) 601–613.
3 Brown-Grant, K. and ter Harr, M.B., Impairment of the control of gonadotrophin secretion after estrogen administration in adult female rats, *J. Endocrinol.*, 73 (1977) 479–505.
4 Chihara, K., Arimura, A. and Schally, A.V., Immunoreactive somatostatin in rat hypophyseal portal blood: effects of anesthetics, *Endocrinology*, 104 (1979) 1434–1441.
5 Clark, R.G., Chambers, G., Lewin, J. and Robinson, I.C.A.F., Automated repetitive microsampling of blood: growth hormone profiles in conscious male rats, *J. Endocrinol.*, 11 (1986) 27–35.
6 Clark, R.G. and Robinson, I.C.A.F., Growth hormone responses to multiple injections of a fragment of human growth hormone-releasing factor in conscious male and female rats, *J. Endocrinol.*, 106 (1985) 281–289.
7 Coifi, P., Croix, D. and Tramu, G., Coexistence of hGHRF and NPY immunoreactivities in neurons of the arcuate nucleus of the rat, *Neuroendocrinology*, 45 (1987) 425–428.
8 Dickson, S.L., Leng, G. and Robinson, I.C.A.F., Stimulus patterning and growth hormone (GH) secretion in male rats, *J. Physiol.*, 452 (1992) 215P.
9 Dyball, R.E.J. and Leng, G., Estimation of stimulus spread from stimulating electrodes in the CNS of the anesthetized rat, *J. Physiol.*, 446 (1991) 68.
10 Fekete, M.I.K., Herman, J.P., Kanyicska, B. and Makara, G.B., Electrical stimulation of the median eminence in rats, changes in catecholamine content and in plasma prolactin and growth hormone concentrations, *Life Sci.*, 27 (1980) 929–933.
11 Fukata, J., Diamond, D.J. and Martin, J.B., Effects of rat growth hormone (rGH)-releasing factor and somatostatin on release and synthesis of rGH in dispersed pituitary cells, *Endocrinology*, 117 (1985) 457–467.
12 Ibata, Y., Okamura, H., Makino, S., Kawakami, F., Morimoto, N. and Chihara, K., Light and electron microscopic immunocytochemistry of GRF-like immunoreactive neurons in the rat hypothalamic arcuate nucleus and median eminence, *Brain Res.*, 370 (1986) 136–143.
13 Kato, M., Suzuki, M. and Kakegawa, T., Modification by hypothalamic lesions of the release of growth hormone (GH) following stimulation of the ventromedial hypothalamic nucleus of the rat, *Brain Res.*, 280 (1983) 69–74.
14 Makara, G.B., Harris, M.C. and Spyer, K.M., Identification and distribution of tuberoinfundibular neurons, *Brain Res.*, 40 (1972) 283–290.
15 Martin, J.B., Plasma growth hormone (GH) resonse to hypothalamic or extrahypothalamic stimulation, *Endocrinology*, 91 (1972) 107–115.
16 Martin, J.B., Brazeau, P., Tannenbaum, G.S., Willoughby, J.O., Epelbaum, J., Terry, L.C. and Druand, D., Neuroendocrine organisation of growth hormone regulation. In S. Reichlin, R.J. Baldessarini, and J.B. Martin (Eds.), *The Hypothalamus*, Raven Press, New York, 1978, pp. 329–357.
17 Meister, B., Hökfelt, T., Vale, W.W., Sawchenko, P., Swanson, L. and Goldstein, M., Coexistence of tyrosine hydroxylase and growth hormone-releasing factor in a sub-population of tuberoinfundibular neurons of the rat, *Neuroendocrinology*, 42 (1986) 237–247.
18 Melander, T., Hökfelt, T., Rökaeus, Å., Cuello, A.C., Oertel, W.H., Verhofstad, A. and Goldstein, M., Co-existence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS, *J. Neurosci.*, 6 (1986) 3640–3654.
19 Millar, R.P., Sheward, W.J. Wegener, I. and Fink, G., Somatostatin-28 is an hormonally active peptide secreted into hypophyseal portal vessel blood, *Brain Res.* 260 (1983) 334–337.
20 Murakami, Y., Kato, Y., Shimatsu, A., Koshiyama, H., Hattori, N., Yanaihara, N. and Imura, H., Possible mechanism involved in growth hormone secretion induced by galanin in the rat, *Endocrinology*, 124 (1989) 1224–1229.
21 Niimi, M., Takahara, J., Sato, M. and Kawanishi, K., Identification of dopamine and growth hormone-releasing factor-containing neurons projecting to the median eminence of the rat by combined retrograde tracing and immunohistochemistry, *Neuroendocrinology*, 55 (1992) 92–96.
22 Ottlecz, A., Samson, W.K. and McCann, S.M., Galanin: evidence for a hypothalamic site of action to release growth hormone, *Peptides*, 7 (1985) 51–53.
23 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press. London, 1982.
24 Poulain, D.A. and Wakerley, J.B., Electrophysiology of magnocellular neurons secreting oxytocin and vasopressin, *Neuroscience*, 7 (1982) 773–808.
25 Renaud, L.P., Neurophysiological organization of the endocrine hypothalamus. In: S. Reichlin, R.J. Baldessarini and J.B. Martin (Eds.), *The Hypothalamus*. Raven Press, New York, 1978, pp. 269–301.
26 Saphier, D.J. and Dyer, R.G., Effects of neonatal exposure to monosodium glutamate on the electrical activity of neurons in the mediobasal hypothalamus, and on plasma concentration of TSH and prolactin, following stimulation of the rostral hypothalamus in adult female rats, *J. Endocrinol.* 89 (1981) 379–387.
27 Tannenbaum, G.S. and Ling, N., The interrelationship between growth hormone (GH)-releasing factor and somatostatin in the generation of the ultradian rhythm of GH secretion, *Endocrinology*, 115 (1984) 1952–1957.
28 Wakerley, J.B. and Lincoln, D.W., The milk ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of parvocellular neurons during oxytocin release, *J. Endocrinol.* 57 (1973) 477–493.
29 Wehrenberg, J.B., Brazeau, P., Luben, R., Bohlen, P. and Guillemin, R., Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor, *Endocrinology*, 111 (1982) 2147–2148.
30 Yeomans, J.S., Practical notes for brain stimulators. In J.S. Yeomans (Ed), *Principles of Brain Stimulation*, Oxford University Press, 1990, pp. 162–179.