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The Short-Term Regulation of Growth Hormone Secretion

by

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A thesis submitted to the School of Graduate Studies of the University of Ottawa in partial fulfillment of the requirements for the degree, Master of Science, in the Department of Physiology, Faculty of Health Sciences.

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DEDICATION

This thesis is dedicated to my parents, Roy MacLean and Shirley Levy Nicholson.

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I wish to express my deepest appreciation for Dr. John S. Cowan's unfailing guidance and support during what was for me an odyssey of discovery. His confidence in my abilities enabled me to explore horizons only dreamed of.

I am indebted to Mary Byers, Ross Layberry and Jack Anthony for their excellent technical assistance, encouragement and support - as well as Miza Davies.

To my family and friends, near and far, who offered me their unqualified support and encouragement, and, in doing so, sustained my dreams, I extend my warmest thanks.

Finally, I wish to thank Anita Bouchard who diligently typed my manuscript, and whose dedication to the task ensured its timely completion.

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Ab	antibody
ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
BSA	bovine serum albumin
cGH	canine growth hormone
c.p.m.	counts per million
CRF	corticotropin releasing factor
g	gravity
GRF	growth hormone-releasing factor
hpGRF	human pancreatic GRF
i.v.	intravenous
MCR	metabolic clearance rate
PBS	phosphate buffer solution
P.E.	polyethylene
rGH	rat growth hormone
RIA	radioimmunoassay
S.E.M.	standard error of the mean
SRIF	somàtostatin
V	apparent distribution volume

ABSTRACT

The interactions between somatostatin (SRIF) and growth hormone-releasing factor (GRF) in the short-term regulation of growth hormone secretion were examined using both a positive and negative somatostatin signal, with the positive signal provided by a somatostatin infusion at two levels; the lower rate was just above the minimum necessary to block all spontaneous bursts, as shown by Cowan et al. (1984), with a slightly higher rate used in the first series of experiments (B series). A clear negative signal was made possible by the availability of a monoclonal antibody (Ab) to somatostatin, which was administered as a bolus i.v. injection in the second series of experiments (M series). Based on evidence provided by the work of Cowan et al. (1984, 1983) as well as somatostatin antisera work in rats (Tannenbaum et al. 1978, Chihara et al. 1983) it was expected that the lowering of endogenous portal somatostatin by the Ab would initiate a growth hormone secretory burst, followed by an elevated baseline. To test the ability of the somatotrophs to respond to GRF in the presence or absence of somatostatin's inhibitory influence, two levels of GRF signal were superimposed on an initial and second control period (when endogenous portal somatostatin may have been high or low due to natural excursions in its release), as well as during the somatostatin infused period and during the post-Ab period. This was intended to elucidate the interactions between somatostatin and GRF in the short-term control of growth hormone.

Fourteen random source adult male mongrel dogs were trained to lie relaxed on a padded table for up to 10 hrs. Blood sampling was normally at 5 min intervals, but was at 2½ min intervals from 10 min before to 15 min following an event (event defined as a GRF injection, the cessation of the somatostatin infusion, or the injection of the monoclonal Ab to somatostatin). All events were 40 min apart. Plasma samples were assayed in triplicate using a double antibody radioimmunoassay for growth hormone.

In 15 experiments there was a response to 26 GRF injections out of a possible 44 (59% response) during the control periods. During the somatostatin infusion period out of a possible 57 responses to GRF, only 1 occurred. There was a total of 17 spontaneous growth hormone secretory bursts during the control periods, resulting in an average interpeak interval of 2.85 hours. In sharp contrast, only 5 spontaneous bursts were observed during the somatostatin infusion period, 4 of which were in one animal. Eleven growth hormone secretory overshoots were initiated by the cessation of the somatostatin signal. The injection of the monoclonal Ab to somatostatin elicited an immediate, large growth hormone secretory response, followed by an elevated baseline in 5 out of 7 cases. During the post-Ab period there were 5 responses to GRF out of a possible 7 (71%), with 2 spontaneous bursts.

The frequency of response to GRF during the somatostatin infusion period vs. the controls and post-Ab period demonstrated the great effect of somatostatin concentration on the sensitivity

of the somatotrophs to GRF. The presence of a post-somatostatin rebound in 11 out of 15 experiments provided indirect evidence for somatostatin playing the major role in initiating the spontaneous growth hormone secretory burst; the direct evidence came from the immediate, large response to the monoclonal Ab to somatostatin. Because of the extreme drop in the sensitivity of the somatotrophs to GRF in the presence of moderate amounts of somatostatin, it appears clear that somatostatin excursions set the timing of growth hormone secretory bursts, while GRF exposure may be the major factor in setting the amplitude of these bursts.

INTRODUCTION

I. Control of Growth Hormone Secretion

The secretion of growth hormone is modified by external stimuli, endogenous neural rhythms (Reichlin 1985), metabolic changes, growth hormone autoregulation and ultrashort feedback of the hypothalamic factors which stimulate and inhibit the release of growth hormone from the somatotrophs (GRF and somatostatin, respectively). Because growth hormone does not have a specific target organ or gland, but exerts its effects on various parts of the body, a consolidated theory of a simple feedback model for its episodic release does not presently exist.

Naturally occurring external stimuli include exercise and stresses, both physical and emotional. These are known to increase growth hormone release in man (Takahashi et al. 1968, Reichlin 1985), while causing inhibition in the rat via a stress-induced increase in somatostatin (see section on "antiserum to somatostatin"). A notable endogenous modification of growth hormone secretion in humans is the surge which occurs 1-2 hours following the onset of deep sleep (Takahashi 1968, Reichlin 1985). Because of the presence of this phenomenon in humans Willoughby et al. (1976) studied the effect of sleep on the episodic secretion of growth hormone in rats, but were unable to find any correlation between the rat growth hormone secretory profile and the sleep phase patterns. In an attempt to find a suitable animal model for human sleep-related growth hormone secretion, Takahashi et al. (1981) used periods of forced

wakefulness in dogs. It was hypothesized that the absence of the growth hormone surge in animals might be due to their polyphasic sleep-waking pattern, in contrast to the monophasic pattern present in humans.

Metabolic and nutritional factors which increase growth hormone secretion include hypoglycemia, the descending limb of blood glucose levels following a carbohydrate rich meal, high protein intake, and certain amino acids (arginine and leucine). Although acute hyperglycemia blocks the stress-induced increase in growth hormone secretion in humans, the chronic hyperglycemia present in diabetes appears to have no effect on growth hormone secretion (Daughaday 1985, Takahashi et al. 1968). Painson and Tannenbaum (1985) examined the effects of intracellular glucopenia in rats, and found it to be a potent inhibitor of growth hormone secretion. The effect on growth hormone was stress mediated however, since the effects were abolished by an antiserum to somatostatin. Although growth hormone is modified by physiologic changes in metabolites their effects are limited, suggesting a primary role for neural mechanisms in the control of growth hormone secretion (Daughaday 1985, Painson and Tannenbaum 1985, Takahashi et al. 1985).

Several approaches have been used to attempt to elucidate the role that growth hormone plays in regulating its own secretion. It was observed by various groups that the central administration of growth hormone (intraventricular, hypothalamus or median eminence), resulted in a marked suppression of growth hormone secretion (Tannenbaum 1980, Richman et al. 1981,

Abe et al. 1983). All work indicated the feedback to be at the level of the hypothalamus, and not the pituitary. A reciprocal relationship was found to exist between hypophysial portal blood somatostatin levels and growth hormone levels in systemic blood (Chihara et al. 1979). Later work by Chihara et al. (1981) showed a significant and dose-related increase of immunoreactive somatostatin in hypophysial portal blood following the central (intraventricular) injection of growth hormone. Molitch and Hlivyak (1980), using a different approach, administered growth hormone to hypophysectomized rats and measured hypothalamic somatostatin, since earlier work had shown a decreased hypothalamic somatostatin content in hypophysectomized animals. As expected, the exogenous growth hormone resulted in a specific increase in hypothalamic somatostatin. Kasting et al. (1981), using a push-pull perfusion technique, found that a rise in the release of immunoreactive somatostatin from the median eminence often coincided with an increase in growth hormone levels in systemic blood, in rats.

Observations showed that various hypothalamic neuropeptides, involved in the release of anterior pituitary hormones, elicited the opposite effect when administered centrally (Lumpkin et al. 1985). This suggested the existence of negative ultrashort feedback for the two hypothalamic factors directly involved in the control of growth hormone release (GRF and somatostatin). Following a study in rats, in which GRF was injected into the 3rd ventricle, Lumpkin et al. (1985) proposed that such a mechanism did exist for GRF. Aquila and McCann (1985) postulated that the

mechanism by which GRF exerts a negative ultrashort loop is by stimulating somatostatin release, since hGRF-40 was found to stimulate somatostatin in a dose-related fashion, in vitro. In keeping with the existence of an ultrashort feedback inhibition, McCann et al. (1984) found that the intraventricular administration of somatostatin resulted in a dramatic elevation of plasma growth hormone.

II. Development of Radioimmunoassay (RIA) for Growth Hormone

Before the development of the radioimmunoassay (RIA) a sensitive method for measuring plasma GH concentration was not available. The standard rat tibial epiphysial plate bio-assay, which gave barely detectable responses with large volumes of plasma, could not be used with single or serial sampling techniques in the same animal (Schalch & Reichlin 1966). In 1964 Schalch & Reichlin reported a double antibody RIA for human growth hormone based on a RIA for insulin. Parker et al. (1965) successfully adapted this assay to the measurement of rat GH, despite the species specificity of GH, using anti-porcine GH serum. With the availability of purified rGH further improvements were made (Schalch & Reichlin 1966). A sensitive radioimmunoassay for canine GH was developed almost a decade later (Lovinger et al. 1974).

III. Episodic Secretion of Growth Hormone

Early investigations on rat GH secretion revealed a wide range of plasma GH concentration, suggesting that GH levels

fluctuated over a period of time. In 1974 Martin, Renaud and Brazeau examined the temporal pattern of GH secretion in adult, conscious, freely behaving male rats. They found that GH secretion in rats was episodic with a mean interpeak interval of just over one hour. A few years later Tannenbaum and Martin (1976) more clearly defined the temporal pattern of GH secretion in the rat. In agreement with the earlier group it was found that rat GH secretion was strikingly episodic, but with a mean peak-to-peak interval of approximately 3.3 hr. It was suggested by Tannenbaum and Martin that the shorter interval arrived at in the first paper was due to taking an average of all interpeak intervals without regard to the double- or triple-peak characteristics of major secretory episodes. The work of the latter group also showed an entrainment of the secretory pattern to the light-dark cycle, although the interpeak interval did not change significantly with constant illumination. Since then many others working with rats have observed a 3-4 hr. interval between GH secretory bursts (Willoughby et al. 1976, S. Eden 1978, 1979, Tannenbaum et al. 1979, G.S. Tannenbaum 1980, Terry and Martin 1981, Wehrenberg et al. 1982, Tannenbaum and Ling 1984, Gurd et al. 1984). Episodic GH secretion in dogs was reported by Takahashi et al. in 1981. Cowan et al. (1984) performed 6 hr. resting control experiments to determine the normal GH secretory pattern in resting, conscious, male dogs. A mean interpeak interval of 3.9 hr. was observed.

The magnitude of the difference between peak burst levels and basal levels is substantial, particularly in the rat with a

range of <1 ng/ml during basal periods, to as high as >600 ng/ml during secretory bursts (Martin et al. 1974, S. Eden 1979, Tannenbaum et al. 1979, Willoughby et al. 1976). The secretion of growth hormone in the dog, while clearly episodic in nature, is not as striking in magnitude as that found in the rat. Cowan et al. (1984) reported a mean basal concentration of 0.8 ng/ml and a mean peak concentration of 5.8 ng/ml in canine plasma growth hormone. It was calculated that just over 50% of all growth hormone secreted was secreted during a secretory burst. Because of the magnitude of the difference between peak and basal levels in rat growth hormone secretion, it stands to reason that far less than 50% of the total rat growth hormone secreted is contributed by basal periods.

IV. Somatostatin; Isolation, Characterization and Synthesis

In their quest for the elusive growth hormone-releasing factor Brazeau et al. (1973) consistently observed that the addition of certain crude hypothalamic extracts to the incubation fluid of dispersed rat pituitary cells in monolayer cultures significantly inhibited the basal secretion of immunoreactive GH by the pituitary cells. The isolation and characterization of this inhibitory factor yielded a 14 amino acid tetradecapeptide, of ovine hypothalamic origin. Both the native and synthetic forms of this somatotropin-release inhibiting factor, known as SRIF or somatostatin, elicited an inhibition of growth hormone secretion in a specific and dose related fashion. In 1974 Brazeau et al. expanded their work using "gentled", nonstressed,

conscious rats and rats anesthetized with sodium pentobarbital. Somatostatin both inhibited and prevented the sodium pentobarbital-induced increase in GH in the anesthetized rats, while significantly depressing the spontaneous secretion of immunoreactive GH in "gentled", nonstressed, conscious rats. In vitro work by Vale et al. (1975) demonstrated that somatostatin could inhibit the secretion of previously synthesized GH as well as newly synthesized GH. It was also shown that somatostatin could block the response to a crude hypothalamic extract with growth hormone-releasing activity, indicating that somatostatin was capable of blocking the expression of the effect on the pituitary of the putative physiological stimulator of GH secretion (Vale et al. 1975). In vivo work by this group, using gentled, unstressed, conscious rats, showed that somatostatin's inhibition of resting plasma GH was of a short duration, which suggested a short half-life of 2-4 minutes, in rats. Schusdziarra et al. (1979) found the half-life of synthetic somatostatin to be 1.82 min, as determined by RIA. The work of Gillioz et al. (1979) which dealt with immunoreactive somatostatin in rat hypophysial portal blood, revealed the existence of a second molecular form of somatostatin. Gel filtration of hypophysial portal plasma yields two immunoreactive peaks, with the major one corresponding to synthetic somatostatin (14 amino acid tetradecopeptide), and the smaller peak representing a larger molecular form. Schally et al. (1976) reported the isolation and structure of porcine hypothalamic somatostatin with a primary structure identical to that of the 14 amino acid

tetradecapeptide ovine hypothalamic somatostatin. They also noted the presence of a second distinct form of somatostatin with a higher molecular weight, which was active both biologically and immunologically. Speiss et al. (1979) isolated and characterized somatostatin from pigeon pancreas. Gel-filtration yielded two molecular forms, with over 99% representing a small species. This predominant form was determined to be identical to ovine and porcine hypothalamic somatostatin in amino acid sequence, structure, biological potency and immunologic activity. The larger molecular form represented .3 to .5% of the gel-filtered somatostatin-like activity. In 1980 Esch et al. determined the primary structure of two larger molecular weight somatostatins, of ovine hypothalamic origin. Both somatostatin-28 and -25 appeared to be NH_2-terminally extended peptides of the tetradeca-peptide somatostatin-14.

V. Antiserum to Somatostatin

Hypothalamic somatostatin has been shown to decrease the release of growth hormone from pituitaries of different species, in vitro (Arimura et al. 1976). If the administration of an antiserum to somatostatin was followed by an increase in growth hormone secretion this would indicate a physiological role for somatostatin. Because of the well known stress-induced decrease in growth hormone secretion in rodents, Arimura et al. (1976) used the rat to investigate the effect of ovine antiserum to somatostatin on basal and post-stress plasma growth hormone

levels. Pretreatment of rats with the antiserum to somatostatin blocked or blunted the stress-induced decrease in plasma growth hormone. From this they concluded that the stress-induced decrease in plasma growth hormone was at least partly due to an increase in the release of somatostatin. Terry et al. (1976) also investigated the mechanism of the stress-induced decrease in plasma growth hormone observed in rats. They found that rats exposed to a mild stress maintained the pulsatile pattern of growth hormone secretion, but with a decrease in peak amplitudes. Rats subjected to a more severe stress, however, showed a complete suppression of pulsatile growth hormone secretion for at least five hours. Pretreatment with antiserum to somatostatin partially restored the pulsatile pattern of growth hormone secretion, although it did not increase basal levels. The authors' interpretation was that while somatostatin had a prominant role in the stress-induced inhibition of growth hormone secretion in the rat, it did not play a physiological role in basal growth hormone secretion. Since this was not found by others working with rats, it is possible that the antiserum itself, or its administration, might have been enough of a stress to evoke additional somatostatin release, over and above that bound by the antiserum. Tannenbaum et al. (1978), having reported earlier that growth hormone secretory bursts were markedly suppressed in response to prolonged food deprivation, assessed the role of somatostatin in the starvation-induced decrease in rat plasma growth hormone. They found that the i.v. administration of antiserum to somatostatin, in 72-hr. food-

deprived rats, immediately restored the high amplitude pulses of growth hormone secretion. In addition they noted a significant elevation of basal growth hormone secretion. Administering this antiserum to normal rats also elicited an elevation in basal growth hormone levels, but did not affect the amplitude of the secretory episodes. Ferland et al. (1976) used an antiserum to somatostatin in conscious, unstressed rats to investigate the physiological role of somatostatin in the normal pulsatile secretion of growth hormone. Administration of the antiserum resulted in elevated basal levels as well as a 2 to 3-fold increase in peak amplitudes, contrary to the work of Tannenbaum et al. (1978) and Terry and Martin (1981). The former finding indicated a physiological role for somatostatin in the control of basal growth hormone secretion. Terry and Martin (1981) did similar work with rats using an antiserum to somatostatin. In agreement with Ferland et al. (1976), they observed a significant increase in basal growth hormone levels following the administration of the antiserum, but with no effect on peak amplitudes. Chihara et al. (1978), using anesthetized rats with basal medial hypothalamic ablation, found that there was no response to the somatostatin antiserum, indicating that it did not act at the pituitary level, but presumably neutralized the somatostatin in the hypophysial portal circulation.

In contrast to the rodent, stress elicits an increase in plasma growth hormone levels in primates (Takahashi et al. 1968). Because the effect of stress on growth hormone secretion in the dog was not documented, V. Chand addressed this in his Master's

Thesis (1983) in conjunction with the use of a sheep antisomatostatin serum. The antiserum, while provoking an immediate, massive growth hormone secretory burst, also caused a 2-15 fold increase in plasma glucocorticoids within 20 minutes of the injection. An injection of goat sera which did not contain antibodies to somatostatin served as a control. Clinical observations and glucocorticoid levels indicated that this control sera was stressful to some degree, with little or no response in growth hormone secretion. The objective in this study was to distinguish between that portion of the antiserum-induced growth hormone secretory burst caused by the antibodies to somatostatin and that which was due to the sera itself, (immunogenic stress). In view of the rat's sensitivity to stress, and the corresponding suppression of growth hormone secretion, it is not surprising that the antisera work with rats produced variable results. In comparing "gentled" (unstressed) and nongentled rats Takahashi et al. (1971) found an inverse relationship between plasma growth hormone and plasma corticosterone levels. Rivier and Vale (1985), working with conscious rats, found that CRF (corticotropin releasing factor), acts centrally to inhibit growth hormone secretion by causing an increase in the release of somatostatin.

VI. Isolation, Characterization & Synthesis; GRF (Somatocrinin)

Up until 1983, hypothalamic somatocrinin had not been isolated or characterized. However, in 1982 Guillemin et al. isolated, characterized, and synthesized three peptides with

growth hormone-releasing activity, obtained from a human pancreatic tumor which had caused acromegaly. The peptides were 44, 40, and 37 amino acid residues, with the latter two having the same sequence as the corresponding initial portion of the 44 amino acid peptide, hpGRF-44; and 30% and 12% of the specific activity of the hpGRF-44 in vitro, respectively. hpGRF-44, the largest peptide, was identical in biological activity both in vitro and in vivo to the still uncharacterized GRF present in hypothalamic extracts (Guillemin et al. 1982). Brazeau et al. (1982) compared the biological activities of these three peptides, hpGRF-44, -40, and -37, with the biological activities of highly purified hypothalamic GRF of porcine or murine origin. All three tumor-derived GRF's, as well as the purified hypothalamic GRF, had identical effects and intrinsic activities, in vitro. The study also suggested that hpGRF-44, which had the highest specific activity (# of biological units/mole), was the primary form of GRF. In 1982 Rivier et al. also reported the isolation and characterization of a 40 amino acid residue with growth hormone-releasing activity, from a human pancreatic tumor (hpGRF-40). Its synthetic replicate had a high potency in stimulating GH release both in vitro and in vivo (Rivier et al. 1982). The first hypothalamic polypeptide with GH-releasing activity to be isolated and characterized was by Speiss, Rivier and Vale (1983). It was a 43-residue polypeptide from rat hypothalamic extracts (rGRF-43), which demonstrated 67% homology with the corresponding N-terminal 43 residues in hpGRF-44, reported earlier by Guillemin et al. (1982). The native

polypeptide and its synthetic replicate did not differ significantly in their biological activities and potencies. The isolation, characterization and synthesis of human hypothalamic somatocrinin, hGRF-44, was reported by Ling et al. in 1984. Not surprisingly the structure of this peptide was identical to that of hpGRF-44, since earlier observations have shown that biologically active peptides produced ectopically by tumors are frequently identical to the products of the physiological source of such peptides (Ling et al. 1984, Guillemin et al. 1982, Imura H. 1980).

VII. Interaction of Somatostatin & GRF in Growth Hormone Release

The antiserum to somatostatin work done by Aminura et al. (1976), Tannenbaum et al. (1978) and others clearly indicated a physiological role for somatostatin in the basal secretion of growth hormone, since the administration of the antisera caused an elevation in basal levels of growth hormone. The lack of effect of the antisera to somatostatin on the pulsatile pattern of growth hormone secretion, however, led many workers to believe that GRF must then be solely responsible for the initiation and amplitude of the growth hormone secretory bursts. With the isolation and characterization of GRF in 1982, a number of studies followed to elucidate the interaction of somatostatin and GRF in the short-term control of growth hormone secretion.

GRF of either hypothalamic or human pancreatic tumor origin was shown to be a potent stimulator of growth hormone release both in vitro (Brazeau et al 1982, Speiss et al. 1983,

Vale et al. 1983) and in vivo (Wehrenberg et al. 1982, Chihara et al. 1985, Tannenbaum and Ling 1984). The response to GRF was found, however, to be markedly variable in vivo. Tannenbaum and Ling (1984) examined the interrelationships of GRF and somatostatin in the generation of the ultradian rhythm of growth hormone secretion in conscious rats. Their results showed that GRF administration during known peak periods caused a marked and immediate increase in growth hormone release, in contrast to little or no response during known trough periods, which was in agreement with the results of others (Wehrenberg et al. 1982, Chihara et al. 1983, Gurd et al. 1984). The striking difference between the two times of GRF administration was abolished by pretreatment with antiserum to somatostatin. Cowan et al. (1985) examined GRF's role in stimulating growth hormone release in dogs. Exogenous GRF administration was superimposed on both control periods, when endogenous somatostatin might be high or low due to natural excursions in its release, and during periods of a moderate somatostatin infusion, just above the rate previously shown to block spontaneous growth hormone secretory bursts. During the control periods more than half of the GRF injections elicited growth hormone responses, comparable in size and duration to spontaneously occurring growth hormone secretory bursts. In sharp contrast, either little insignificant growth hormone responses, or no response at all was seen in response to the GRF administered during the somatostatin infused period. This strongly suggested a decreased pituitary sensitivity to GRF in the presence of physiological concentrations of somatostatin.

Sheppard et al. (1985) using an *in vitro* system of perifused somatotrophs found that somatostatin consistently and completely blocked GRF-induced growth hormone release. This supported previous *in vitro* work by others (Brazeau et al. 1982, Speiss et al. 1983, Vale et al. 1983). Both the *in vivo* and *in vitro* work indicated that when both stimulatory and inhibitory hypothalamic factors were present, the inhibition predominated.

In vitro work by Stachura (1976 & 1977), and Cowan et al. (1983) showed that the removal of a somatostatin signal was consistently and immediately followed by a rebound release of growth hormone, suggesting a physiological role for somatostatin in setting the timing of growth hormone secretory bursts. *In vivo* evidence for somatostatin's role in initiating secretory bursts came from the somatostatin antiserum work, with the administration of antiserum eliciting an immediate growth hormone secretory burst, followed by an elevated baseline (Plotsky and Vale 1985, Ono et al. 1984, Chihara et al. 1983, Tannenbaum et al. 1978). This suggested a tonic inhibition by somatostatin on the somatotrophs during basal growth hormone secretion, with the removal of the inhibition initiating a secretory burst.

The *in vitro* work by Stachura (1976 & 1977) and Cowan et al. (1983) indicated that somatostatin, while playing a major role in initiating a secretory burst, influenced the size of the burst as well; with the size of the post-somatostatin rebound greater following a longer period of somatostatin inhibition. This supported the hypothesis proposed by Stachura, that during somatostatin inhibition only the release of growth hormone is

inhibited, with growth hormone synthesis continuing, resulting in the accumulation of a readily releasable pool of growth hormone. However, the existence of intracellular feedback for growth hormone synthesis was indicated, since not all of the growth hormone secretion suppressed by exposure to somatostatin was recovered in the burst following the removal of the somatostatin signal by Cowan et al. (1983).

While GRF was shown to be capable of initiating a growth hormone secretory burst (provided the level of endogenous somatostatin was low), its major role was unclear. In 1986 Kraicer et al. used an _in vitro_ system of perifused somatotrophs to further elucidate GRF's major role in growth hormone secretion. It was found that including GRF in the perfusate for the duration of the somatostatin signal resulted in an average 8.7 fold increase in the size of the post-somatostatin overshoot. Continued application of GRF after removal of the somatostatin signal, while increasing further the size of the burst, did not influence the amplitude but resulted in a longer duration. This suggested that _in vivo_ GRF plays a major role in the accumulation of the readily releasable pool of growth hormone and therefore in the amplitude of the ensuing burst, following a decrease in endogenous somatostatin.

The overall tonic effect of GRF on growth hormone secretion is well established. This was demonstrated by Wehrenberg et al. (1982) who used a monoclonal Ab (antibody) to GRF in rats, which resulted in the abolition of pulsatile growth hormone secretion. The authors' interpretation was that episodic pulses of growth

hormone secretion appear to be due to a positive drive by GRF; not noted by the authors, however, was the concomitant lowering of basal growth hormone levels, shown in the data. This then demonstrated the importance of GRF's tonic effect on growth hormone secretion, rather than a specific role in initiating growth hormone secretory bursts. The work of Ono et al. (1984) further supported this, using a GRF antiserum in conjunction with somatostatin antiserum (rats). The peak growth hormone secretory response normally seen following somatostatin antiserum administration was reduced 67% by pretreatment with GRF antiserum. Further work showed that somatostatin antiserum-induced growth hormone release was suppressed by the GRF antiserum in a dose-related fashion.

VIII. Physiological Context

The availability of a pure monoclonal antibody to somatostatin made possible a clear negative signal *in vivo*, allowing for an experimental design with both positive and negative somatostatin signals; superimposing GRF injections during both the somatostatin infused period and the post-Ab period was intended to elucidate the interactions between somatostatin and GRF in the short-term control of growth hormone secretion. More specifically, it would test the ability of the somatotrophs to respond to GRF in the presence or absence of somatostatin's inhibitory influence. Responses to such antibodies are clearly separated from any response to immunogenic stress because of the trivial mass of material administered;

this was not possible in the earlier work with antisera to somatostatin because of the substantial foreign protein content of the serum itself.

The use of the dog as the experimental model was appropriate because of the close similarity to man in growth hormone responses and the dog's adaptation without stress to handling and the experimental conditions. This was important to eliminate any effects of stress on growth hormone secretion, which can be substantial. As can be seen in "Results", clinical observations and glucocorticoid data indicated the Ab itself was not stressful.

The dog was also able to provide the blood volume necessary for frequent serial sampling over a period of nine hours. The choice of male animals was to avoid any variation due to reproductive cycles in females. In addition, there are post-pubertal age-related changes in growth hormone secretion in female rats (S. Eden 1979) which may also be the case in the canine species.

IX. General Objective

To more clearly elucidate the interactions between somatostatin and GRF in the short-term regulation of growth hormone secretion, with an emphasis on the initiation and control of growth hormone secretory bursts.

X. Specific Questions:

(i) Does a drop in endogenous somatostatin alone trigger a GH

secretory burst?

(ii) How dependent is the sensitivity of the somatotrophs to GRF on the amount of somatostatin present in the milieu, either before or during GRF application?

(iii) Why do excursions in GRF alone not reliably stimulate growth hormone secretion; consequently, what is the role of GRF in naturally occurring GH bursts?

(iv) Somatostatin appears to set the timing of naturally occurring bursts by declining to a "critical threshold"; is this its only major role, or does it influence the size of the burst as well?

MATERIALS AND METHODS

ANIMAL MODEL

Fourteen random source adult male mongrel dogs weighing 12.2-23.5 kg were used for seventeen experiments. This weight range was chosen in order to permit normalization of data and valid comparisons with earlier publications on canine growth hormone work. Dogs underwent a minimum three week period of conditioning, consisting of a health examination, evaluation of nutritional status and hematological profiles, treatment of internal and external parasites as required, and vaccination against rabies, canine parvo-virus, canine distemper-hepatitis, kennel cough and leptospirosis.

Housing was in standard cages under conditions of constant temperature (20-23°C) and relative humidity (45-55%) on a 12 hour light and 12 hour dark cycle with lights on at 6:00 a.m.. Diet was comprised of dry dog food and water ad libitum, in addition to canned dog food on the days in which training occurred. The animals received routine daily exercise in outdoor runs.

Dogs were trained over a one to two week period to lie relaxed on a padded table for ten hours, during which time they were exposed to the sound of the centrifuge and infusion pump. At the end of each training period they were rewarded with a can of dog food.

EXPERIMENTAL & SURGICAL PROCEDURES

On the day of the experiment animals were released from their cages into outdoor runs by 7:15 a.m. A short period later

they were brought in and weighed before being moved upstairs to the experimental room for surgery. Surgery was completed by 8:30 a.m. An exception to this was the approximately 45 min later start with the two animals used in the pilot study on the monoclonal antibody to SRIF (SOMA #8).

Surgery consisted of saphenous vein to inferior vena cava and cephalic vein cannulations with Clay Adams PE205 and PE190 tubing, respectively. This was performed under sterile conditions. The local anesthetic used was lidocaine hydrochloride. Surgical areas were shaved the day before the experiment to minimize stress on the experimental day. The cannulae were flushed with weakly heparinized isotonic saline (1:50) to confirm and maintain patency until the experiment was begun. The saphenous cannula was used for blood sampling and administering GRF and antibody injections, and the cephalic was used for the SRIF infusion. There was a minumum waiting period of 40 min between the completion of surgery and the first blood sample.

Blood samples of 1.2 ml were taken to obtain 0.7 ml of plasma for determination of canine growth hormone. An additional 3 ml of blood was taken at specified times for glucocorticoid assay, which was used as an indicator of stress. Sampling was at 2.5 min intervals from 10 min preceeding to 15 min following an event; with an event comprising either a GRF injection, initiation or termination of the somatostatin infusion, or an injection of monoclonal antibody to somatostatin. Fluid replacement was with isotonic saline, approximately one and a

half times the blood sample volume, or with weakly heparinized isotonic saline as required.

Changes observed in either animal behaviour or experimental procedure were recorded on the protocol sheet. Sterile removal of the cannulae was performed and a furacin dressing applied. Animals were then returned to their cages and given a can of dog food.

EXPERIMENTAL DESIGN

I. B Series; The Effect of Somatostatin on Pituitary Sensitivity to GRF

The purpose of these experiments was to determine the effect of physiological concentrations of somatostatin on pituitary sensitivity to GRF. Conscious resting dogs received two levels of bolus intravenous (i.v.) injections of GRF during periods in which either endogenous levels of somatostatin were unmanipulated, the so-called control periods, or during an infusion of somatostatin, which was within the physiological range.

a) B1 and B2 In two experiments (B1 and B2), in the same animal, an i.v. infusion of somatostatin at a steady rate of 0.25 $\mu g\ kg^{-1}\ min^{-1}$ was initiated 40 min prior to the first blood sample. This rate was just above that previously shown to block spontaneous growth hormone secretory bursts (Cowan et al. 1984). The infusion was continued for the first 200 min of blood sampling. Four bolus injections of GRF were administered i.v. during the somatostatin infusion at 40 min intervals (2 at each

level of 125 ng/kg and 500 ng/kg). At 200 min the somatostatin infusion ended, followed by a second control period with two more bolus injections of GRF. All events were 40 min apart, with blood sampling ending 40 min after the sixth GRF injection.

b) B3-B8 In the remainder of the experiments in the B series (B3-B8) there was an initial control period of 85 min designated as "pre-somatostatin", (when endogenous levels of somatostatin were unmanipulated). During this time 2 bolus i.v. injections of GRF were administered at 40 min intervals (one each at 500 ng/kg and 2000 ng/kg). Forty minutes after the second GRF injection the somatostatin infusion was begun (0.25 $\mu g\ kg^{-1}\ min^{-1}$ in B3-B6), with a duration of 205 min. Forty-five minutes after the initiation of the somatostatin infusion the first of 4 bolus i.v. injections of GRF was administered; the others followed at 40 min intervals (2 at each level). The somatostatin infusion was followed by a second control period, referred to as "post-somatostatin", with two more bolus injections of GRF at 40 min intervals, giving a total of 8 GRF injections. Sampling ended 40 min after the last GRF injection. This design was used in 6 experiments in 4 animals (B3-B6; for details see Tables I-III). In 2 experiments (B7 and B8) a slightly lower rate of somatostatin was infused (see footnote to Table I). This lower rate is nonetheless still known to be effective in blocking spontaneous bursts of growth hormone release in dogs (Cowan et al. 1984).

II. The Effect of Somatostatin on Pituitary Sensitivity to GRF Using a Monoclonal Antibody to Somatostatin

To further elucidate the role of somatostatin on the pituitary's sensitivity to GRF a monoclonal antibody (Ab) was used to diminish free endogenous somatostatin.

a) Monoclonal Ab to Somatostatin (SOMA #8); Pilot Study

Blood was sampled at 5 min intervals for 240 min in two conscious resting dogs. At 240 min they received a bolus i.v. injection of monoclonal Ab to somatostatin. For 30 min following the injection the sampling interval was reduced to 2.5 min to increase the resolution of any response. Blood sampling then continued for an additional 210 min at 5 min intervals. The monoclonal Ab used in these 2 experiments was SOMA #8 (J.C. Brown), and was later shown to have a lower affinity *in vitro* for somatostatin and to be less effective *in vivo* than SOMA #10.

b) Monoclonal Ab to Somatostatin (SOMA #10); M Series

The monoclonal Ab designated as SOMA #10 (J.C. Brown) was used in 7 experiments on 7 dogs. These experiments were similar to the B series with the last 120 min period, containing 2 bolus injections of GRF, serving as a second control period. Following this second control period a bolus i.v. injection of monoclonal Ab to somatostatin was administered. This was followed by two GRF injections 40 min apart, resulting in a total of 10 GRF injections (5 each at 500 ng/kg and 2000 ng/kg). The somatostatin infusion rate for this series was 0.15 $\mu g\ kg^{-1}\ min^{-1}$ (as in B7 and B8, and as justified by Cowan et al. 1984).

ORIGIN & CHARACTERISTICS OF EXOGENOUS MATERIAL

a) GRF

The GRF (1-44) peptide was purchased from Bachem Inc. in powder form and stored at 4°C. The day before the experiment an amount sufficient for one experiment was weighed on a Chan gram electrobalance for preparation of the concentrated solution. On the day of the experiment the concentrated solution was made up by dissolving the GRF in 100 ml of water, 100 mg BSA, 0.0198g L-ascorbic acid sodium salt and 57.5 λ N glacial acetic acid solution, to give a concentration of 1100 ng/ml per kg, and kept on ice. In the 10 min preceding an injection either 50 λ (small injection), or 200 λ (large injection) was diluted to 1.1 ml with isotonic saline. One ml was administered i.v. with a disposable syringe for an injected dose of 500 ng/kg or 2000 ng/kg.

b) Somatostatin

Synthetic cyclic somatostatin-14 was purchased from Sigma Chemical Co. (catalogue #S-9129) as a solid, with the addition of 2 ml of saline resulting in a 2.5 µg/λ stock solution, which was stored at -12°C. Fifteen minutes before the somatostatin infusion began a calculated amount of concentrate was diluted in 20 ng/ml lactose in saline solution, with a total volume of 10 ml, and placed in a 10 ml glass syringe, which was used in the Harvard Compact Infusion Pump.

c) Monoclonal Antibody (Ab) to Somatostatin

The monoclonal Ab to somatostatin was kindly provided by Dr. J.C. Brown, Dept. of Physiology, University of British Columbia. The antibody designated as SOMA #8 was in a freeze dried form.

It was partially dissolved in 0.5% BSA in phosphate buffer solution (PBS) the day before the first pilot study, with the unused portion stored at -70°C until the second pilot experiment.

Two batches of the monoclonal antibody designated as SOMA #10 were received in ascites fluid, containing approximately 6 ng/ml of immunoglobulin. The first batch was used in dogs 1-3, and the second was used in dogs 4-7. This was injected i.v. as a bolus ranging from 12.31 λ/kg to 30 λ/kg, (Table II).

HANDLING OF SAMPLES

Blood samples were placed in labelled, heparinized, 1.5 ml polypropylene Eppendorf test tubes, kept on ice. They were spun in pairs in an Eppendorf micro centrifuge (12,800 G) for 3 min, at 4°C. Time from collection of blood sample to centrifuging did not extend much beyond 5 min. Plasma was pipetted into labelled 1.5 ml polypropylene Eppendorf test tubes and stored at -12°C until the end of the experiment. Plasma samples were then stored at -70°C.

Plasma samples for canine growth hormone (cGH) assay were packed in dry ice (-78.5°C) and sent to Dr. J. Kraicer's lab, where they were once again stored at -70°C until assay. On the day of assay samples were allowed to thaw at room temperature.

Samples for glucocorticoid assay were handled in the same fashion, with the exception of being pipetted into labelled glass counting vials after centrifuging. On the day of assay samples were removed from the -70°C deep freeze and allowed to thaw at room temperature.

GROWTH HORMONE DOUBLE ANTIBODY RADIOIMMUNOASSAY

Plasma GH was measured, usually at one dilution, using a double-antibody radioimmunoassay method, similar to that of Lovinger et al. (1974). The anti-cGH (AFP-214-121577; raised in rhesus monkey) and the antigen (AFP-1983-B; highly purified canine pituitary GH) were obtained from Dr. A.F. Parlow, Director, Pituitary Hormones and Antisera Center, Torrance, California. The cGH was used as the standard and for iodination. The goat antiserum to rhesus monkey gamma globulin (Calbiochem #539873, lot 493082) and the rhesus monkey serum used as carrier (Calbiochem #566611, lot 486074) were purchased from Behring Diagnostics, San Diego, California.

Iodination of purified canine GH was performed as follows: 15 mg chloramine T (Baker E494-6) was dissolved in 5.0 ml 0.5M phosphate buffer, pH 7.6. About 10 µg cGH was accurately weighed on a Cahn microbalance and dissolved in 0.01M NaHCO to a concentration of 1.0 µg/µl. To this was added 50 µl 0.5M phosphate buffer and the resultant solution was transferred to the shipping vial containing 1 mCi NaI-125 (Dupont Canada, NEZ-033H). The weighing pan was rinsed with a further 50 µl phosphate buffer, and the rinse transferred to the same vial. Ten microliters chloramine-T solution was then added to initiate the reaction. Following 30 sec gentle mixing with a pasteur pipet, the reaction mixture was transferred to a 22 x 0.8 cm Sephadex G50 fine column, previously rinsed with 1 to 2 ml 15% BSA and equilibrated at room temperature with at least 30 ml

degassed 0.05M barbital buffer, pH 8.6 Collection of forty 8 drop (about 0.3 ml) fractions into tubes containing 100 µl 15% BSA in PSB, pH 7.6 was begun immediately; fractions 8 to 40 were placed on ice as collected. When collection was complete, radioactivity was determined in 10 µl samples from each fraction using a Searle Model 1195 Gamma Spectrometer, resulting in an elution profile with 2 "peaks" of radioactivity, a major void volume "peak" containing the labelled cGH and a minor one containing unreacted I-125. Five to six fractions containing the labelled cGH were pooled and 100 or 200 µl aliquots were snap frozen on dry ice and stored at -80°C for up to 4 months before preparation of a new lot.

Rechromatographed labelled cGH was prepared on the day of use by chromatography of an aliquot on a column of Sephadex G-100 fine, under the conditions described above. This chromatography results in three "peaks" of radioactivity with the immunoreactive cGH predominantly in the central one. Fractions from the column eluate were pooled as required.

The binding affinity for each fresh batch of labelled cGH was assessed by incubating dilutions of anti-cGH with 10,000 cpm of rechromatographed labelled cGH in the absence of unlabelled cGH. The dilution of antibody which gave a specifically bound/total radioactivity ratio of about 0.33 was used routinely. This usually corresponded to a final, tube dilution of 1:1,600,000.

Assay tubes routinely contained 100 µl standard or plasma, 100 µl antibody dilution, 100 µl labelled cGH and 300 µl buffer.

(1% BSA in 0.01M phosphate buffered saline containing 0.025M EDTA, pH 7.6), all added on day 1 of a 5 day incubation at 4°C. In the morning of day 4, appropriately diluted precipitating antibody (goat anti-rhesus gamma globulin) and carrier rhesus serum were added (100 µl of each). On day five, 2 ml of cold buffer without BSA were added, followed immediately by 75 min of centrifugation at 1550g in an MSE Coolspin II refrigerated centrifuge. The supernatants were decanted and discarded, and radioactivity was determined in the retained precipitates using an LKB 1272 Clinigamma spectrometer. Standard curves and quality control samples were placed at the beginning and end of each assay. All standards and samples were assayed in triplicate.

Results were expressed as ng of cGH (AFP-1983-B) per ml of plasma. Practical sensitivity of the assay was 0.5 ng/ml. Inter-assay and intra-assay coefficients of variation were 10.32% and 2.14% respectively over the period of this research, based on repetitive measurements of a pooled plasma quality control sample. This pool was chosen arbitrarily to give a bound/bound in reference ratio of about 0.5. The coefficients of variation were calculated using the method of Rodbard and Jaffe (1979).

GLUCOCORTICOID ASSAY

Plasma glucocorticoid was measured in duplicate using a modified version of the fluorometric method of Silber, Busch and Oslapas (1958). All glassware was rinsed in absolute ethanol and air-dried before use. Standard solutions of No H-4001

hydrocortisone (Sigma Chem. Co.) and No C-2505 corticosterone (Sigma Chem. Co.) were made to a concentration of 100 mg% in absolute ethanol. Each was diluted 1:500 with isotonic saline, yielding a final concentration of 2 µg/ml. 500 λ standards of each and a 500 λ blank (distilled water) were placed at the beginning and end of each assay.

200-500 λ of each sample was pipetted in duplicate into labelled 15 ml centrifuge tubes. Sample volume was brought up to 500 λ with distilled water, as required. 6 ml of methylene chloride was added to each tube by an automatic dispenser followed by shaking 60 times to ensure mixing. The tubes were then centrifuged at 2000 rpm for 3 min to define the aqueous and organic layers. The aqueous layer was aspirated. Two ml aliquots of the methylene chloride layer were transferred into the corresponding-labelled steroid tubes and stoppered immediately. Two ml of a mixture of 65% concentrated H_2SO_4 and 35% absolute ethanol was added to each tube from an automatic dispenser, in sets of 4, followed by shaking for 20 sec. Sets of 4 tubes were repeated every 1½ min. The top layer (methylene chloride) was aspirated and the samples were read exactly 60 min after shaking, using an Aminco-Bowman Spectophotofluorometer. The excitation wavelength was 465 µ and the emission wavelength was 525 µ.

A linear regression program was used on a Wang 600 minicomputer to calculate apparent cortisol concentration using the cortisol standard readings. These were converted to mixed glucocorticoid on the assumption that the ratio of cortisol to

corticosterone is approximately 1:1 (Ganong 1983).

$$[\text{Plasma glucocorticoid}] = [\text{Plasma cortisol}] \times \frac{(F - 0) \times 2}{(B - 0) + (F - 0)}$$

F = reading of 2 µg/ml cortisol std

B = reading of 2 µg/ml corticosterone std

0 = Background fluorescence

PROCESSING OF DATA

a) Continuous Secretion Rates

A single compartmental first approximation previously validated for ACTH (Cowan 1978) was used to convert bivariate time vs. concentration data to continuous secretion rates. The values used for metabolic clearance rate (MCR) and apparent distribution volume (V) of growth hormone were those arrived at in previous work by Cowan et al. (1984). Growth hormone was found to have a MCR approximately half of that of ACTH so the model should work even better, since its slower turnover would decrease the error of the model. The equation used was as follows:

$$\text{Secretion Rate} = (MCR \cdot GH_t) + \left(V \cdot \frac{d(GH_t)}{dt}\right)$$

where MCR = 3.99 ± 0.30 ml kg^{-1} min^{-1}

V = 57.9 ± 5.5 ml/kg

b) Integrated Peak Area

The size of a peak was determined from the integrated area of the peak minus the basal secretory rates (ng kg^{-1} min^{-1}). The integrated area was computed on the Wang 600-14-TP minicomputer as sums of histogram rectangles topped by right triangles (Cowan et al. 1984). Data are presented as Means ± Standard error of the mean ($\bar{x}$ ± S.E.M.).

c) Statistics

Statistical comparisons were made using analysis of variance (ANOVA) where appropriate with the Wang 600-14-TP minicomputer and commercially available software, (volumes 133 and 139 of "Wang's Calculator Assisted Statistics Series", 1972). The unpaired Student's t-test was used for those statistical comparisons in which the null hypothesis was tested for a single variable between two groups or treatments.

RESULTS

RESPONSE TO hpGRF (1-44) DURING CONTROL PERIODS; B SERIES AND M SERIES

In 15 experiments a total of 56 graded bolus injections of hpGRF (1-44) were administered i.v. during the "control" periods, (pre-somatostatin and post-somatostatin), during which time the level of somatostatin in portal blood was entirely endogenous, and the concentration may have been high or low. A clear response to these 56 events would only have been possible in 44 cases; in 12 cases the injection occurred while some other GH excursion was still in progress. These obscuring influences were either spontaneously occurring bursts or a long response to the preceeding event. There was a response to 26 of these 44 GRF injections (59%), with the integrated extra area (peak-basal) (ng/kg) and duration (min) comparable to spontaneously bursts occurring during the same time period ($p < .05$) (Tables IV-VI). In the remaining 18 cases either small, statistically insignificant excursions in growth hormone secretion or no response at all was seen.

The possible dependence of the size of the response on both dose of GRF, and on occurring before or after somatostatin infusion was tested by 2-way ANOVA. The size was not dependent on either of these factors ($p < .05$). Nonetheless, when responses from assymetrical experiments were added in (experiments in which responses only occurred either before or after somatostatin, but not both), then an unpaired t-test of all 26 burst sizes (ng/kg)

before and after somatostatin showed that GRF-induced bursts before somatostatin were larger than after somatostatin ($0.01 < p < 0.005$). This is probably just the effect of increased "n".

Neither the dose of GRF nor the period in which the responses occurred (pre-somatostatin vs post-somatostatin) caused a significant difference in the duration of the responses ($p < .05$).

In order to answer the following 2 questions a 2-way ANOVA was performed:

1) Is the likelihood of obtaining any response greater in response to the larger dose of GRF?

2) Is the likelihood of obtaining any response to GRF greater or smaller in the pre-somatostatin period or the post-somatostatin period?

No significant difference was found ($p < .05$) in the frequency of responses to GRF based on either the dose of GRF or the temporal position, with respect to the somatostatin infused period.

RESPONSE TO GRF DURING SRIF INFUSION; B SERIES AND M SERIES

The first six experiments of the B series (B1-B6) involved somatostatin infusion rates of 0.25 $\mu g\ kg^{-1}\ min^{-1}$. B7, B8 and the seven experiments in the M series (1-7) were at a slightly lower rate of 0.15 $\mu g\ kg^{-1}\ min^{-1}$. Since no significant differences in the effect of the infusions on basal growth hormone concentration were seen, resulting from the different

rates of somatostatin infused, the results from all fifteen experiments were pooled. It is worth noting that the lower rate of somatostatin infusion was sufficient in Cowan et al. (1984) to block spontaneous growth hormone bursts.

A total of 60 bolus injections of hpGRF (1-44) (30 at each dose level) were administered i.v. during the somatostatin infused periods in fifteen experiments. To these 60 injections there was one response, to the last large injection, in experiment B4. It was small in size (150.3 ng/kg) and of a short duration (12.5 min) when compared to the spontaneously occurring bursts in the control periods (376.2 ± 42.3 ng/kg, 33.6 ± 2.3 min, n=7), or to the GRF-induced bursts in the control periods (379.4 ± 99.8 ng/kg, 26.2 ± 2.2 min, n=26).

Thus, of 57 possible responses to GRF injection during somatostatin infusion (3 injections of the 60 occurred during spontaneous bursts and so were not available for possible responses), there was only one small, short response to GRF (1.75%), clearly indicating a massive effect of modest amounts of somatostatin on pituitary sensitivity to GRF.

SPONTANEOUS BURSTS DURING CONTROL PERIODS; B SERIES AND M SERIES

During the control periods of all 15 experiments, 17 spontaneous bursts occurred, yielding an average frequency of 1/2.85 hr (Table VII). The average size and duration was 376.2 ± 43.2 ng/kg and 33.6 ± 2.3 min, respectively (Table VIII). Neither the size nor the frequency of the spontaneous bursts were different in the two control periods (pre-somatostatin vs post-somatostatin).

SPONTANEOUS BURSTS DURING SRIF INFUSION; B SERIES AND M SERIES

In fifteen experiments there was a total of 5 spontaneous bursts during the somatostatin infused period (ave. frequency = 1/10.22 hr.). Four of the 5 occurred in one dog during two experiments. These were smaller than during control periods, with an average integrated area of 156.9 ± 32.6 ng/kg and a duration of 20.5 ± 3.9 min. All 5 occurred during the latter part of the somatostatin infusion, between 27 and 90 minutes before the end of a 205 min infusion. Thus except in one dog, these SRIF infusion rates were sufficient to block almost all bursts.

POST-SOMATOSTATIN REBOUND; B SERIES AND M SERIES

In the B-series, in all 6 experiments with the higher rate of somatostatin infusion (0.25 $\mu g kg^{-1} min^{-1}$), the removal of the somatostatin signal resulted in a growth hormone secretory overshoot, with an average lag of 9.2 min in 3 cases of 6, and no discernable lag in the remaining 3. In the 9 experiments with the slightly lower somatostatin infusion of 0.15 $\mu g\ kg^{-1}\ min^{-1}$ there were 5 post-somatostatin rebounds.

The size of the post-somatostatin overshoot was not dependent on the level of somatostatin infused (404.7 ± 122.1 ng/kg vs 897.4 ± 285.7 ng/kg following 0.25 $\mu g\ kg^{-1}\ min^{-1}$ and 0.15 $\mu g\ kg^{-1}\ min^{-1}$, respectively) (Table IX). The size of the post-somatostatin overshoots, although large except in two cases (B1 and B2), was not significantly different from either the

spontaneously occurring bursts or the GRF-induced bursts occurring in the control periods (by a 2-way ANOVA, $p < .05$); there was, however, a tendency for post-somatostatin overshoots to be larger than spontaneous bursts (628.7 ± 157.9 ng/kg vs. 376.2 ± 43.2 ng/kg, $p < .10$ by Student's t-test).

IMMEDIATE RESPONSE TO ANTIBODY (Ab) INJECTION; M SERIES

The monoclonal antibody (Ab) to somatostatin (SOMA #10) was administered i.v. at 410 minutes, following the post-somatostatin control period, in 7 experiments (1-7). Two of the 7 Ab injections did not work, possibly due in one case to the quantity used (M4) since in that case, through an error, an unknown quantity was used. In the other case (M6) there was no apparent reason for the failure to respond, except that the animal in question was a low GH secretor with minimal responses to GRF and no spontaneous bursts. In the remaining 5 Ab experiments administration of the Ab resulted in an immediate, large growth hormone secretory burst, followed by an elevated baseline.

The size of the response to the Ab was consistently large (1734.2 ± 682.2 ng/kg; range 572.7 - 4389.2 ng/kg) (Table X). Although the average size of the immediate response to the Ab was substantially larger than the average size of the spontaneous bursts or GRF-induced bursts during control periods, three spontaneous bursts and three responses to exogenous GRF during the control periods were within this range.

POST-Ab RESPONSE TO GRF (1-44); M SERIES

In the 5 experiments with an immediate growth hormone response to Ab there were 10 bolus injections of hpGRF (1-44) following the Ab injection. A clear response to these 10 events was only possible in 7 cases, due to obscuring in 3 cases by either spontaneously occurring bursts or a long response to the preceeding event. Of these 7 there was a response to 5 GRF injections (71.4%). The size of these growth hormone secretory bursts was not significantly different from the GRF-induced bursts in the post-somatostatin period ($p < .20$) (Tables IV-VI).

POST-Ab SPONTANEOUS BURSTS; M SERIES

In the 5 successful Ab experiments 2 spontaneous bursts occurred in the post Ab period. One was small in size and the other was large. This gave a spontaneous burst frequency of 1/5 hr (1/3.65 hr if the time of the average response to the Ab is excluded) (Tables VII and VIII).

Because of the number of responses to exogenous GRF and the few spontaneous bursts during the relatively short post-Ab period, further statistical comparisons of frequency or size were not appropriate.

SOMATOSTATIN AND BASAL GROWTH HORMONE CONCENTRATION

In 15 experiments the basal growth hormone concentration ranged from 0.2-2.0 ng/kg (pre-Ab periods), although it was very consistent within an experiment. (Table XI). The first two experiments in the B series (B1 & B2) differed slightly in

experimental design in that they did not contain an initial control period, but began with the somatostatin infusion. This left a total of 28 control periods (pre-somatostin and post-somatostatin). During these control periods basal growth hormone secretion was not always present or sustained for any appreciable length of time. This was as expected since endogenous somatostatin was unmanipulated, with low endogenous levels resulting in either spontaneous bursts or responses to exogenous GRF. In addition, a post-somatostatin overshoot was occurring in the initial portion of the post-somatostatin period in 11 out 15 experiments. Because the period following the peak level of a burst may yield a basal secretory rate before basal concentration is obtained basal concentration was used, rather than basal secretory rates . Only 5 experiments yielded precise basal growth hormone concentrations in both control periods, but an additional 6 experiments did so in one control period. Hence an unpaired Student's t-test was used to compare the two groups. No significant difference was found ($t=.2$, $n=16$), indicating that there was neither a time-dependent shift nor a post-somatostatin effect on basal growth hormone concentration.

A 1-way ANOVA was used to compare first control period basal growth hormone concentration data, second control period data, and those in the somatostatin infused period, with no significant differences found among them. This clearly indicated that both levels of the somatostatin infusion (0.15 $\mu g\ kg^{-1}\ min^{-1}$ and 0.25 $\mu g\ kg^{-1}\ min^{-1}$) did not further lower basal growth hormone secretion, supporting earlier work by Cowan et al. (1984) in

which a somatostatin infusion of 0.15 μg kg^{-1} min^{-1} was used.

Similarly, pooling of data from the two control periods (justified by their demonstrated equivalence, see above) yielded, in a t-test against data during SRIF infusion, a value of t (n=17), showing clearly no effect of SRIF on basal GH.

By way of reinforcement, and to avoid possible mismatching of data which might occur if test groups did not represent the same animals, the 5 experiments in which steady basal GH concentrations were obtained in both control periods and during SRIF infusion were subjected to 2-way ANOVA (period vs animal). There was no significant difference among periods ($p > .2$); not unexpectedly, however, there was a significant difference between animals ($.01 < p < .05$).

POST-Ab BASAL GROWTH HORMONE CONCENTRATION

All five Ab injections which elicited an immediate response were followed by an elevated basal GH concentration, (2.7 ± 0.6 fold increase) (Table XI). In addition, in one of the two experiments in which the Ab injection did not elicit an immediate response (M6), basal GH concentration was subsequently increased by a factor of 1.5.

A 2-way ANOVA was performed on the five successful Ab experiments to test for a significant difference between pre-Ab basal GH concentration and post-Ab basal concentration. A significant difference was found between the pre- and post-Ab basal GH concentration ($p < .001$) as well as between animals ($p < .002$).

GLUCOCORTICOID DATA

Glucocorticoid levels ranged from undetectable, in relation to the background fluorescence, to just over .8 µg/dl in a total of 15 experiments (Tables XII and XIII). Although there were fluctuations in glucocorticoid levels during the course of an experiment they could not be clearly related to external manipulations; notably, the antibody injection could not be shown as a stress. A small rise in glucocorticoid levels could, in some cases, be traced to observations of animal behaviour.

These data suggest that no aspect of these experiments in conscious dogs is stressful.

DISCUSSION

I. CONTROL PERIODS; RESPONSE TO GRF

It is well known that rats exhibit a GH ultradian rhythm, entrained to the light-dark cycle (Tannenbaum and Martin 1976). In work done with rats GRF was shown to be a potent stimulator of GH release during known peak periods, with little or no response seen to exogenously administered GRF during basal periods (Tannenbaum and Ling 1984, Wehrenberg et al. 1982). This difference in response to GRF with respect to time was abolished by antisera to somatostatin, which strongly suggested a decreased ability of the somatotrophs to release extra GH in response to GRF in the presence of physiological levels of somatostatin.

Dogs also exhibit an episodic pattern of GH secretion, but with no apparent entrainment to the light-dark cycle (Takahashi et al. 1981, Cowan et al. 1984). In the present experimental design the initial control period was needed for comparisons by animal, with the second control period to check for a time shift or post-somatostatin differences. During these so-called control periods endogenous portal somatostatin may have been high or low due to natural excursions in its release. The positive response to only just over half (59%) of the GRF injections during the control periods was therefore as expected, since presumably the somatotrophs' ability to respond to, or their sensitivity to GRF was dependent on the level of endogenous portal somatostatin.

IIa) SOMATOSTATIN SETS THE TIMING OF SPONTANEOUS GROWTH HORMONE SECRETORY BURST; INDIRECT EVIDENCE

Cowan et al. (1984) found that 6 hour infusions of a moderate amount of somatostatin (0.15 μg kg^{-1} min^{-1}) abolished all spontaneous growth hormone secretory bursts, in dogs, but did not diminish basal growth hormone release. This strongly suggested that while there is a relative excess of somatostatin acting on the somatotrophs during basal secretion, since extra somatostatin does not further lower basal secretion, but there must be very little present during a growth hormone secretory burst, since a modest extra amount prevents such a burst.

In the present experiments there was a total of 5 spontaneous bursts during the somatostatin infused period, in 15 experiments. Four of the 5 occurred in one dog during 2 experiments. The average integrated area of the peak for the 5 bursts was small, with an atypically short duration, which may indicate that while the level of somatostatin was not sufficient to entirely block spontaneous bursts during part of the infused period, it was high enough to influence the sensitivity of the somatotrophs to GRF. A similar observation may have been made in the rat. It is interesting that Terry et al. (1976) observed that rats exposed to a mild stress maintained their pulsatile pattern of growth hormone secretion, but with a decrease in peak amplitude. Because the rat responds to stress with increased somatostatin (unlike man and dog) this decrease in peak amplitude may have been due to increased somatostatin.

As well, it is tempting to speculate on the existence of

somatostatin-somatostatin feedback during the somatostatin infusion which is also supported by evidence related to rebounds. Such feedback would presumably lower endogenous portal somatostatin over time; eventually the combination of low endogenous portal somatostatin and the infused exogenous somatostatin may not have exceeded the threshold for the 2 animals involved, although all 5 bursts occurred with the higher level of somatostatin (0.25 μg kg^{-1} min^{-1}). It is interesting to note that all 5 bursts occurred towards the end of the somatostatin infusion (between 27 and 90 min before the end of a 205 min infusion), which would be consistent with this speculation but also raises the question of whether or not it becomes easier to break through somatostatin's tonic inhibition as time goes on.

Implicitly, then, a "critical threshold" must be reached in the decline in somatostatin to initiate a growth hormone secretory burst. The decreasing level of somatostatin before this threshold is reached, probably has very little effect on growth hormone secretion, with maybe a small upward shift in basal growth hormone concentration. The in vitro work of Cowan et al. (1983) supports this; in that study the application of a somatostatin signal to perifused rat somatotrophs maximally suppressed growth hormone secretion, and the removal of the signal was consistently followed by a major overshoot of growth hormone, within one minute.

IIb) SOMATOSTATIN SETS THE TIMING OF THE SPONTANEOUS GROWTH HORMONE SECRETORY BURST; DIRECT EVIDENCE

Earlier work with rats and dogs using antisera to somatostatin supported a physiological role for somatostatin in initiating the growth hormone secretory burst. Initial somatostatin work by Chihara et al. (1978) and Tannenbaum et al. (1978) contained evidence that the administration of somatostatin antiserum initiated a growth hormone secretory burst, although it was not noted or interpreted by the authors at that time. V. Chand (1983), using a sheep somatostatin antiserum, found that the administration of the antiserum provoked an immediate, massive growth hormone secretory burst, in all 5 cases in dogs. Because of the foreign protein content of the sera, the response to the antibody could not be clearly separated from the control response to immunogenic stress.

The availability of a pure monoclonal Ab to somatostatin provided a clear negative signal; the monoclonal Ab provided a signal which was free of any obscuring effect of immunogenic stress on growth hormone secretion, which had not been possible with the conventional somatostatin antisera because of the foreign protein content of the serum itself. That the injection of monoclonal Ab was not discernably stressful is supported by the glucocorticoid data (see Results). The immediate post-Ab period therefore constituted a mirror image of somatostatin infusion. It was hypothesized that the monoclonal Ab would be able to bind enough endogenous portal somatostatin to result in the decline of the available somatostatin concentration to the

"critical threshold", which would then presumably initiate a growth hormone secretory burst. It was considered possible that there would be another necessary condition, specifically the presence of enough GRF in the portal blood up to that point to have stimulated the accumulation of a readily releasable pool of growth hormone.

In this study the design of M series (1-7) included a bolus injection of monoclonal Ab to somatostatin at 410 min, following the post-somatostatin "control period". Two of the 7 Ab injections did not elicit an immediate growth hormone secretory burst; in experiment M4 the failure to do so was presumably due to an inadequate quantity of Ab (see Results). The failure of the Ab to provoke an immediate secretory response in M6, however, might have been due to other factors, since this animal was a low growth hormone secretor with no spontaneous bursts present during the 530 minute experiment, and minimal responses to the exogenous GRF, (one small response in each control period). The absence of an immediate growth hormone response to the Ab might then have been due to either the presence of an unusually high level of endogenous somatostatin, which presumably would not have been sufficiently bound by the Ab to result in a decline in free endogenous somatostatin to the critical threshold, or not enough GRF present to stimulate the accumulation of a readily releasable pool of growth hormone. Indeed, it might have been a combination of both. It is worth noting, however, that the post-Ab basal growth hormone concentration was 1.5 times the pre-Ab control concentration in this animal. For this same parameter there was

no significant rise in M4 and an increase to 2.7 ± 0.6 fold in the remaining 5 successful Ab experiments.

In the 5 successful Ab experiments the administration of the Ab resulted in the immediate (t > 2.5 min) initiation of a large growth hormone secretory burst (range 572.7-4389.2 ng/kg). These 5 large responses to the Ab raise the question of why spontaneous bursts, as well as the responses to exogenous GRF are not this large, since the somatotrophs are clearly capable of a greater response than normally occurs spontaneously or with GRF administration.

III) POST-SOMATOSTATIN REBOUND

Various hypothalamic neuropeptides involved in the release of anterior pituitary hormones have been shown to elicit the opposite effect when administered centrally (Lumpkin et al. 1985), indicating the existence of ultrashort feedback. In agreement with this, McCann et al. (1984) found that the intraventricular administration of somatostatin resulted in a dramatic elevation of plasma growth hormone.

In the present experiments it was hypothesized that the frequency of the post-somatostatin rebound would be a good indicator of somatostatin-somatostatin feedback. If one speculates that exogenous somatostatin would be expected to provide feedback to the hypothalamus, it would then be predicted that a decreased release of endogenous somatostatin into the portal circulation would result. With the cessation of infusion of the exogenous somatostatin its circulating concentration might

fall faster than the endogenous supply would rise, and the circulating level of portal somatostatin might then be below the "critical threshold", which would result in the initiation of a growth hormone secretory burst. The short half life in dogs of exogenous somatostatin (1.82 min, Schusdziarra et al. 1979), would make this more likely.

Eleven post-somatostatin growth hormone secretory overshoots were seen following the removal of the somatostatin signal, in a total of 15 experiments. In the 6 experiments with the higher level of somatostatin infusion (0.25 $\mu g\ kg^{-1}\ min^{-1}$; B1-B6), there was an average lag of 9.2 min in three cases, with no discernable lag (lag < 2.5 min) in the remaining 3. In the 9 experiments with the lower rate of somatostatin infusion 5 post-somatostatin rebounds occurred, with no discernable lag between the removal of the somatostatin signal and the initiation of the overshoot (0.15 $\mu g\ kg^{-1}\ min^{-1}$; B7, M2, M5-M7). It is worth noting that all experiments with the slightly higher rate of somatostatin infusion contained a post-somatostatin rebound, whereas only just over half (56%) of the experiments at the lower rate of somatostatin infusion did so, which may indicate that the lower rate of somatostatin infusion was not sufficient to reduce the level of endogenous somatostatin to below the "critical threshold" in all animals. The probability of the 11 spontaneous bursts occurring by chance in the 10 min period following the infusion was less than 3.34×10^{-12}.

In a total of 15 205 min somatostatin infusions there were 5 spontaneous and 1 GRF-induced growth hormone secretory bursts.

All 6 growth hormone excursions occurred towards the end of the somatostatin infusion, which raises the question of whether it becomes easier to break through somatostatin's inhibition as time goes on and may also support the hypothesis that somatostatin-somatostatin feedback is occurring.

The size of the post-somatostatin growth hormone secretory overshoot was not influenced by the rate of somatostatin infusion used, though it has been shown in vitro (Cowan et al. 1983) that the duration of the infusion does influence the size of the overshoot.

IV) SPONTANEOUS GROWTH HORMONE BURSTS; CONTROL PERIODS VS. SOMATOSTATIN INFUSION PERIOD

Cowan et al. (1984) performed resting control experiments in conscious dogs, which consisted of taking plasma samples for 6 hours at 5 min intervals. A spontaneous burst frequency of 1/3.9 hr, was obtained. In the present study a shorter interpeak interval was seen during the control periods, 2.85 hours. This discrepancy is not large and indeed is substantially less than the inter-animal variation.

As noted in results the average integrated area of the peaks, as well as the average peak duration of these spontaneous bursts were not different in the two control periods, confirming that these spontaneous bursts were not significantly altered by the preceeding 205 min somatostatin infusion.

During the somatostatin infusion spontaneous growth hormone bursts were not expected to occur, because of a decreased

sensitivity of the somatotrophs to GRF in the presence of a moderate amount of somatostatin, as predicted by the earlier work of Cowan et al. (1984, 1985). However, in a total of 15 experiments there were 5 spontaneous bursts during this period. Not surprisingly, they had both a much smaller integrated area and a shorter duration than those occurring during the control periods (for discussion see "Somatostatin Sets the Timing of the Growth Hormone Burst" and "Post-Somatostatin Rebound" sections of discussion).

V) SOMATOSTATIN AND THE SIZE OF THE GH SECRETORY BURST

Evidence for somatostatin influencing the size of the growth hormone secretory burst came from the in vitro work of Stachura (1976, 1977) and Cowan et al. (1983). Stachura applied 20 min and 40 min pulses of somatostatin to perifused rat pituitary explants, with rebound release occurring immediately following the removal of the somatostatin signal. From this work Stachura proposed that somatostatin blocked the release of growth hormone from the somatotrophs, while growth hormone synthesis continued, resulting in the accumulation of a "readily releasable pool" of growth hormone. Cowan et al. (1983) applied 10 min and 60 min signals of somatostatin to perifused rat somatotrophs, with the removal of the somatostatin signal consistently followed by the initiation of a major overshoot of growth hormone, within one minute. The indication for somatostatin playing a role in the size of the growth hormone burst was that the size of the post-somatostatin growth hormone secretory overshoot was greater after

the longer somatostatin signal. Contrary to the work of Stachura, however, it was found that not all of the growth hormone secretion suppressed by exposure to somatostatin was recovered in the post-somatostatin rebound, indicating that intracellular feedback exists for growth hormone synthesis. This difference in results might be due to both the cruder in vitro preparation used by Stachura (tissue pieces), and greater duration of the somatostatin signals used by Cowan et al. (1983), with a 60 min interval being closer to the physiological time course, based on the frequency of spontaneously occurring bursts in both dogs and rats. Perhaps, then, a 20 min or 40 min somatostatin signal was not sufficient to evoke substantial intracellular feedback on growth hormone accumulation.

Based on the earlier in vitro work it was therefore not unexpected that the average peak integrated area of the 11 post-somatostatin rebounds, following the 205 min somatostatin infusion, showed a tendency to be larger than the spontaneous bursts that occurred during the control periods, (628.7 ± 157.9 ng/kg vs. 376.2 ± 43.2 ng/kg, $p < .10$ by the Student's t-test). The long preceeding period of inhibition by somatostatin would have presumably resulted in the accumulation of a somewhat larger readily releasable pool of growth hormone than would have accumulated during the considerably shorter periods between release events during the rest of the design.

VIa) POST-Ab RESPONSE TO GRF

Earlier work in rats using antisera to somatostatin showed

GRF to be a potent stimulator of growth hormone release; with the difference in response to exogenous GRF between known trough and peak periods abolished by the administration of somatostatin antiserum (see Introduction "Antiserum to Somatostatin). In the present study it was expected that there would be an increased frequency of response to the GRF injections following Ab administration when compared to that during the control periods, because of the somatotrophs increased sensitivity to GRF in the relative absence of free endogenous somatostatin. Such was the case with a response to just over 71% of the GRF injections during this period, (5/7 possible) compared to 59% during the control periods, though the "n" is too small here to show statistical significance.

It was hypothesized that the "readily releasable pool" of growth hormone would not accumulate to the same extent without the tonic inhibition by somatostatin; this may be why there was not an even higher response to the exogenous GRF. It may be that in the 2 cases in which a response to the GRF could not be separated from noise, there was not a large enough pool of growth hormone in the immediately releasable form, since in both cases these were the small GRF injections following the GH secretory response to the Ab injection. The diminished extent of accumulation of the "readily releasable pool" of growth hormone may also account for why the 5 detectable responses to the GRF injections were of a relatively small integrated area.

VIb) POST-Ab PERIOD; SPONTANEOUS GROWTH HORMONE SECRETORY BURSTS

During the relatively short post-Ab period, in the 5 successful Ab experiments, 2 spontaneous growth hormone secretory bursts occurred. This was a burst frequency of 1/3.65 hr when correction is made for the durations of the immediate response to the Ab injection. Because of the small "n" meaningful comparisons could not be made with growth hormone secretory events from earlier periods.

VII) SOMATOSTATIN AND BASAL GROWTH HORMONE CONCENTRATION

The earlier work with rats using antisera to somatostatin established a physiological role for somatostatin in the control of basal growth hormone secretion. Tannenbaum et al. (1978), as well as others found that administering an antiserum to somatostatin to stressed rats not only blocked the stress-induced decrease in growth hormone secretion, but resulted in a significant elevation of basal growth hormone secretion. The administration of the somatostatin antiserum to unstressed rats also resulted in a marked elevation of basal growth hormone levels (Tannenbaum et al. 1978, Terry and Martin 1981). Because the administration of the somatostatin antiserum resulted in an increase in growth hormone secretion a tonic inhibition of the somatotrophs by somatostatin was indicated.

Cowan et al. (1984) administered moderate amounts of exogenous somatostatin, in 6 hour infusions, to conscious, resting dogs. The level of somatostatin used was just sufficient to abolish all spontaneous secretory bursts, but did not lower

basal growth hormone secretion rates. This indicated that during basal growth hormone secretion the somatotrophs are already responding maximally to a relative surfeit of somatostatin, and cannot readily further lower basal growth hormone secretion.

In the present experiments the same level of somatostatin was infused as that in Cowan et al. (1984) in all 7 experiments in the M series, as well as in the last 2 experiments of the B series, (B7 and B8), (0.15 $\mu g\ kg^{-1}\ min^{-1}$). In the first 6 experiments of the B series a slightly higher rate was used (0.25 $\mu g\ kg^{-1}\ min^{-1}$). Statistical analysis, as well as visual assessment of the basal growth hormone data from both control periods (pre- and post-somatostatin), and the somatostatin infused period clearly showed that there was no significant effect of the somatostatin infusion, at either level, on basal growth hormone secretion. This supported the earlier work by Cowan et al. (1984), which had suggested a relative excess of somatostatin acting on the somatotrophs during basal growth hormone secretion.

VIII) POST-Ab BASAL GROWTH HORMONE CONCENTRATION

Based on somatostatin's role (see above) in the control of basal growth hormone secretion, the injection of monoclonal Ab to somatostatin was expected to be followed by an elevated "basal" growth hormone secretion. This post-Ab basal secretion was expected to lie between normal basal levels and peak burst levels. In the 5 successful Ab experiments, in which there was an immediate growth hormone secretory response, basal growth

hormone concentration following the burst was increased by an average factor of 2.7 ± 0.6 in the post-Ab period, (range 2.0-6.6 fold increase). This was significantly higher than pre-Ab basal growth hormone concentration, as tested by 2-way ANOVA. In addition, in one of the 2 experiments in which there was not an immediate and marked secretory response to the Ab, post-Ab basal growth hormone concentration was increased by a factor of 1.5; indicating that while the Ab did not bind enough endogenous portal somatostatin to result in a decline in somatostatin to the critical threshold, a sufficient amount was bound to decrease the influence of somatostatin on the somatotrophs' release of growth hormone. This suggests that a decline in somatostatin has only a modest effect on growth hormone secretion before the critical threshold is reached, with perhaps a small upward shift seen in basal growth hormone secretion.

IX) GRF's ROLE IN GROWTH HORMONE SECRETION

The *in vivo* work of Wehrenberg et al. (1982) demonstrated GRF's overall tonic effect on growth hormone secretion. The administration of a monoclonal Ab to GRF in rats resulted in a dramatic lowering of basal growth hormone levels, in addition to the abolition of pulsatile growth hormone secretion.

The extent to which the "readily releasable pool" of growth hormone accumulates appears to be where GRF plays its major role in the growth hormone secretory burst. This is supported by the *in vitro* work of Kraicer et al. (1986). Using a system of perifused rat somatotrophs it was found that the peak secretion

rate of the post-somatostatin overshoot was increased by a factor of 8.7 ± 0.39 (S.E.M.) by including GRF in the perfusate for the duration of the somatostatin signal, resulting in secretion rates comparable to those seen in vivo during a growth hormone secretory burst. Maintaining GRF in the perfusate following the removal of the somatostatin signal did not further increase the peak secretion rates although it did increase the duration of the overshoot. Comparing the durations of the in vitro overshoots to those seen in vivo in dogs suggested that GRF present during the secretory burst probably had very little influence on the magnitude of that burst. This is supported by the present study, in which the size of the integrated peak area of GRF-induced growth hormone secretory bursts during the control periods is not dependent on the dose of GRF (small vs. large injection). Thus, GRF's major role appears to be setting the amplitude of the growth hormone secretory burst by stimulating the accumulation of a "readily releasable pool" of growth hormone during the period before the burst.

During basal growth hormone secretion, when moderate amounts of somatostatin are impinging on the somatotrophs, GRF has little or no immediate effect on growth hormone secretion. This was shown with work in rats, in which the administration of GRF during known trough periods elicited either no response in growth hormone secretion or little insignificant excursions (Tannenbaum and Ling 1984, Chihara et al. 1983, Wehrenberg et al. 1982).

In the present study bolus injections of GRF administered during the control periods, when endogenous portal somatostatin

may have been high or low, elicited growth hormone secretory responses just over half of the time, with integrated peak area and duration comparable to spontaneously occurring bursts during the same periods. In marked contrast only 1 out of a possible 57 responses to GRF occurred during the somatostatin infused period, in a total of 15 experiments, with a small peak integrated area and short duration, (see "Somatostatin Sets the Timing of the Growth Hormone Secretory Burst" and "Post-Somatostatin Rebound"). This further supports the view that the ability of the somatotrophs to respond to GRF is dependent on the amount of somatostatin impinging on the somatotrophs, with the growth hormone releasing action of GRF blocked in the presence of moderate amounts of somatostatin.

X) INTERACTION OF SOMATOSTATIN AND GRF

The present study provides both indirect and direct evidence that somatostatin initiates the spontaneous growth hormone secretory burst by declining to a "critical threshold". The indirect evidence was a) effect of somatostatin infusion on response to GRF and b) the presence of post-somatostatin rebound following the termination of the somatostatin signal, while the direct evidence was the immediate growth hormone secretory response to the monoclonal Ab to somatostatin, with a drop in endogenous somatostatin alone initiating a large growth hormone secretory burst. Excursions in GRF alone, however, have been shown to be unreliable in stimulating growth hormone secretion, although both *in vitro* and *in vivo* studies have shown GRF to be a

potent stimulator of growth hormone secretion. Studies in rats using antisera to somatostatin, as well as the response to GRF in the different periods of the present study have strongly suggested that the ability of the somatotrophs to respond to GRF is dependent on the amount of somatostatin present. For a review of the mechanisms of action of somatostatin and GRF at the cellular level see Sheppard et al. (1985), Kraicer and Chow (1982) and Hall et al. (1986). It is, however, very interesting to note that in the present study the frequency of bursts in response to exogenous GRF was higher than that of spontaneous bursts, during the control periods. This strongly suggests that as the level of somatostatin decreases the somatotrophs are able to respond to our relatively large exogenous doses of GRF even though the "critical threshold" has not been reached for a spontaneous burst. Hence, when significant levels of somatostatin are present, inhibition of growth hormone secretion predominates.

CONCLUSION

In the present study an infusion of a moderate amount of somatostatin abolished all but 6 growth hormone secretory events, in a total of 15 experiments. Had no somatostatin been infused, by analogy to control periods, 52 events (GRF-induced or spontaneous) would have occurred (see "Somatostatin Sets the Timing of the Growth Hormone Secretory Burst; Indirect Evidence). This strongly suggested that while there is a relative excess of somatostatin acting on the somatotrophs during basal growth hormone secretion there must be very little present during a growth hormone secretory burst; this implies that a "critical threshold" must be reached in the decline in somatostatin to initiate a growth hormone secretory burst. The direct piece of evidence in support of a drop in endogenous somatostatin alone triggering a growth hormone secretory burst was provided by the clear negative signal possible with the monoclonal Ab to somatostatin, with an immediate (< 2.5 min), large growth hormone secretory burst in response to the Ab.

The role of GRF in initiating a growth hormone secretory burst was examined by superimposing GRF injections during the different experimental periods. The frequency of response to GRF during the control, somatostatin infused and post-Ab periods showed that the ability of the somatotrophs to respond to GRF with growth hormone release was dependent on the amount of somatostatin present. Hence, an excursion in GRF alone cannot stimulate growth hormone secretion when moderate amounts of somatostatin are present.

In the present study the size of the response to GRF was not dose dependent, which was not unexpected, based on the *in vitro* work of Kraicer et al. (1986). It was found in that study that including GRF in the perifusate for the duration of the somatostatin signal resulted in a massive increase in the readily releasable pool of growth hormone available for immediate release upon disinhibition, but that continuing GRF application after removal of somatostatin did not further increase the peak amplitude of the burst. It did however result in an increased duration, which when compared to the duration of *in vivo* growth hormone secretory events, suggested that the continuing presence of GRF, once a secretory event has begun, does not influence the size of the growth hormone secretory burst *in vivo*. Thus, GRF's major role in the spontaneously occurring growth hormone secretory burst appears to be influencing the extent to which the readily releasable pool of growth hormone accumulates, and therefore the amplitude of the ensuing burst.

Although GRF appears to be the major determinant of the amplitude of the growth hormone secretory burst, somatostatin may influence the size of the burst as well. This was indicated by the *in vitro* work of Cowan et al. (1983), with longer tonic inhibition of the somatotrophs by somatostatin allowing the accumulation of a larger readily releasable pool of growth hormone. It was therefore not surprising that the peak integrated area of the post-somatostatin rebounds following a long period of inhibition in the present study had a tendency to be larger than that of the spontaneous or GRF-induced growth hormone secretory bursts during the control periods.

Figure 1a) Growth hormone plasma concentration vs. time in experiment B1.

FIG 1a

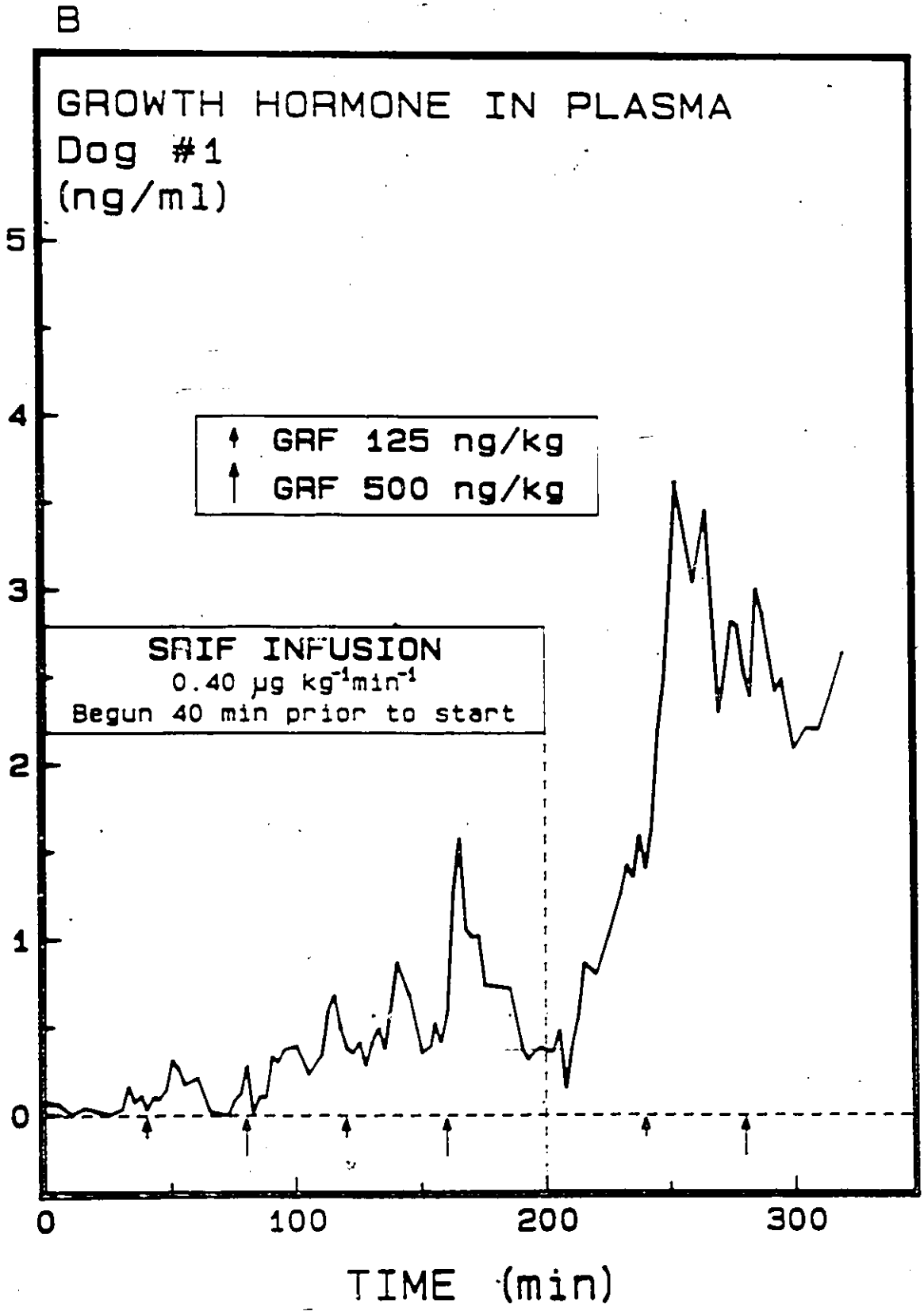

B
GROWTH HORMONE IN PLASMA
Dog #1
(ng/ml)
5
4
3
2
1
0
GRF 125 ng/kg
GRF 500 ng/kg
SRIF INFUSION
0.40 μg kg⁻¹min⁻¹
Begun 40 min prior to start
0
100
200
300
TIME (min)

Figure 1b) Growth hormone plasma concentration vs. time in experiment B2.

FIG 1b

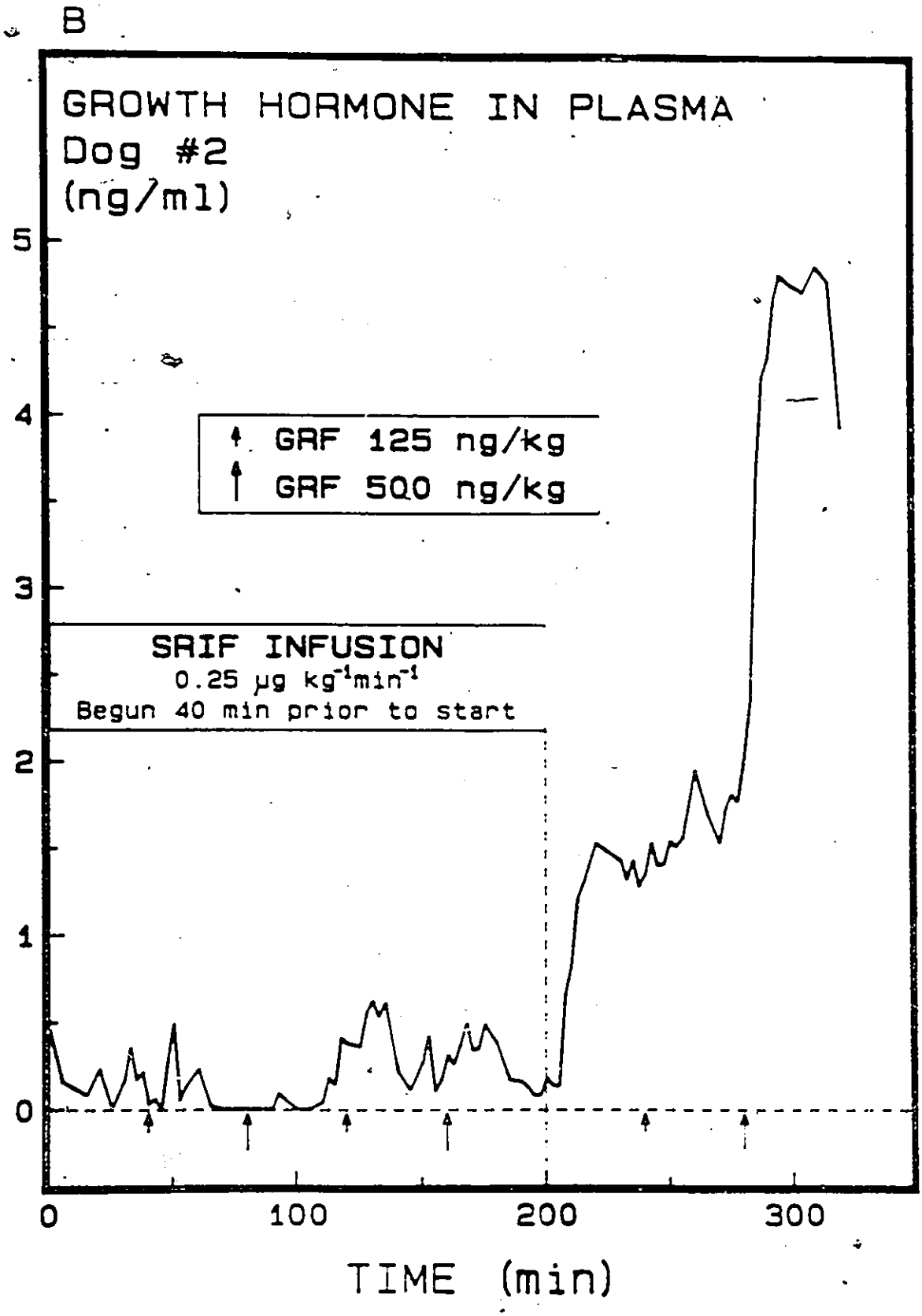

B
GROWTH HORMONE IN PLASMA
Dog #2
(ng/ml)
5
4
3
2
1
0
GRF 125 ng/kg
GRF 500 ng/kg
SRIF INFUSION
0.25 μg kg⁻¹min⁻¹
Begun 40 min prior to start
0
100
200
300
TIME (min)

Figure 1c) Growth hormone secretion rates vs. time in experiment B1. In experiments B1 and B2 the somatostatin infusion was initiated 40 min prior to the first blood sample, and continued for the first 200 min of blood sampling. A post-somatostatin rebound was present in both cases following the cessation of the infusion, with a 10 min lag in B1 and no discernable lag in B2, (sampling interval 2.5 min).

FIG 1c

B

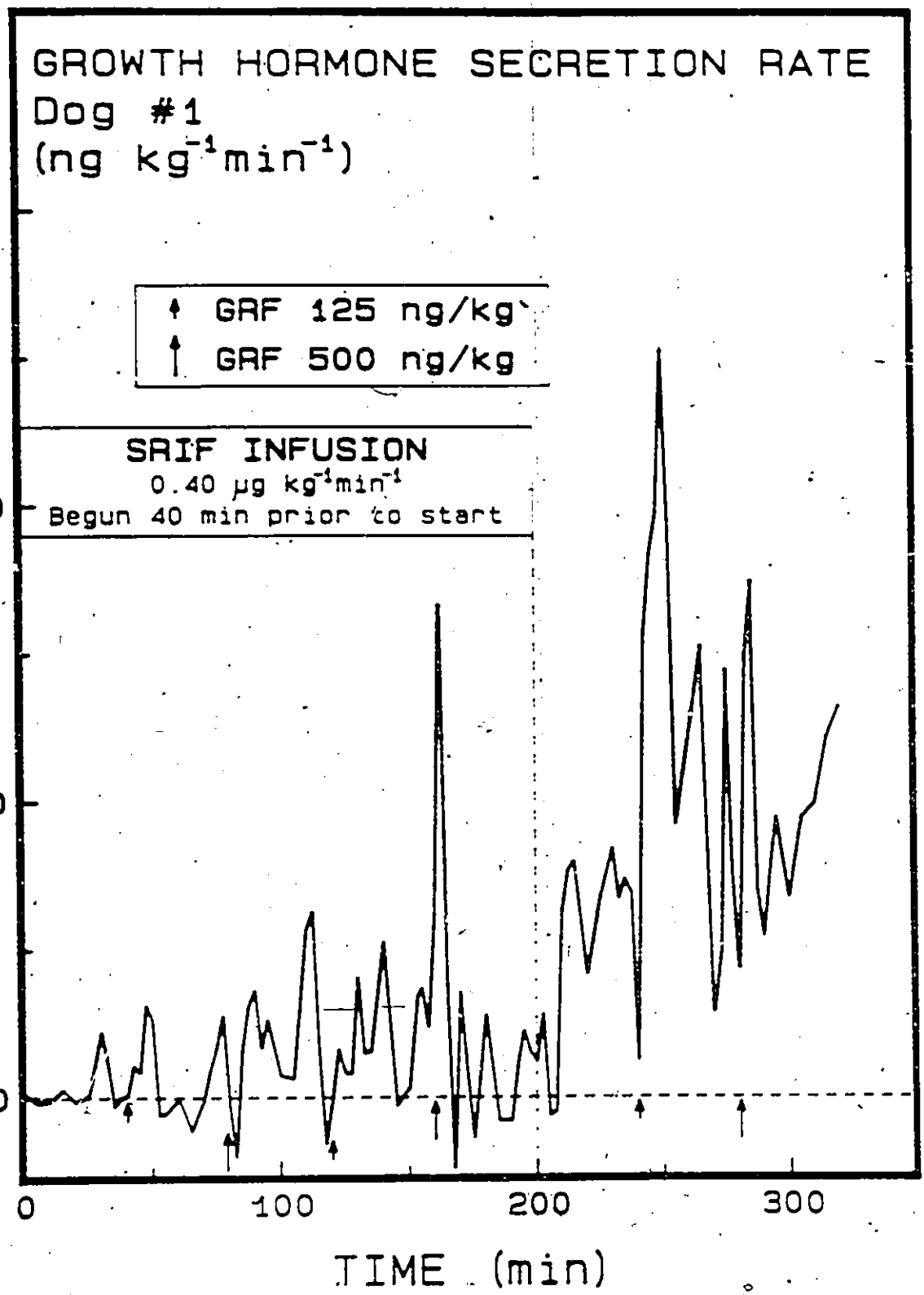

GROWTH HORMONE SECRETION RATE
Dog #1
(ng kg⁻¹min⁻¹)
GRF 125 ng/kg
GRF 500 ng/kg
SRIF INFUSION
0.40 μg kg⁻¹min⁻¹
Begun 40 min prior to start
30
20
10
0
0
100
200
300
TIME (min)

Figure 1d) Growth hormone secretion rates vs. time in experiment B2.

FIG 1d

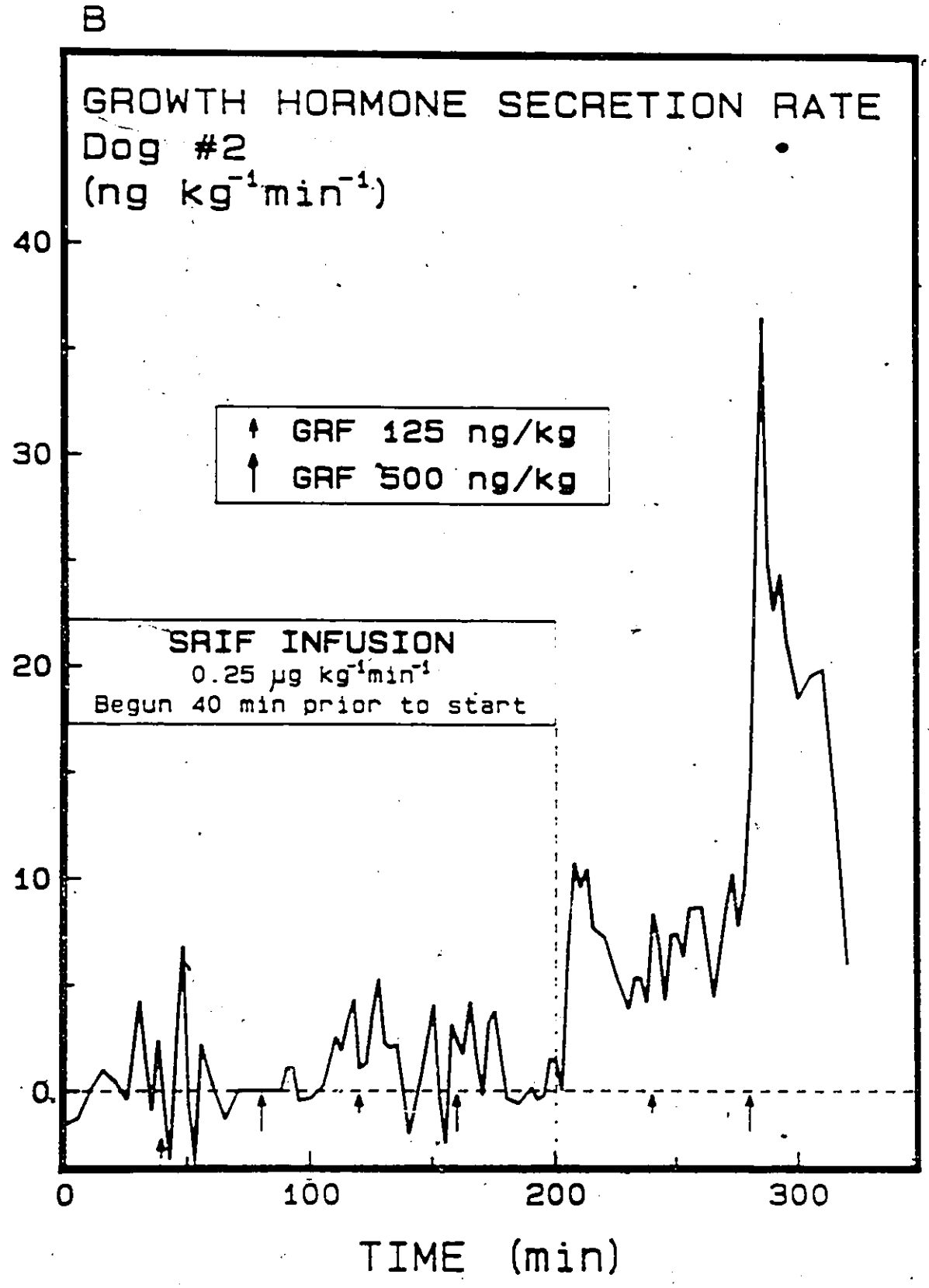

B
GROWTH HORMONE SECRETION RATE
Dog #2
(ng kg⁻¹min⁻¹)
40
30
20
10
0
GRF 125 ng/kg
GRF 500 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
Begun 40 min prior to start
0
100
200
300
TIME (min)

Figure 2a) Growth hormone plasma concentration vs. time in experiment B3.

FIG 2a

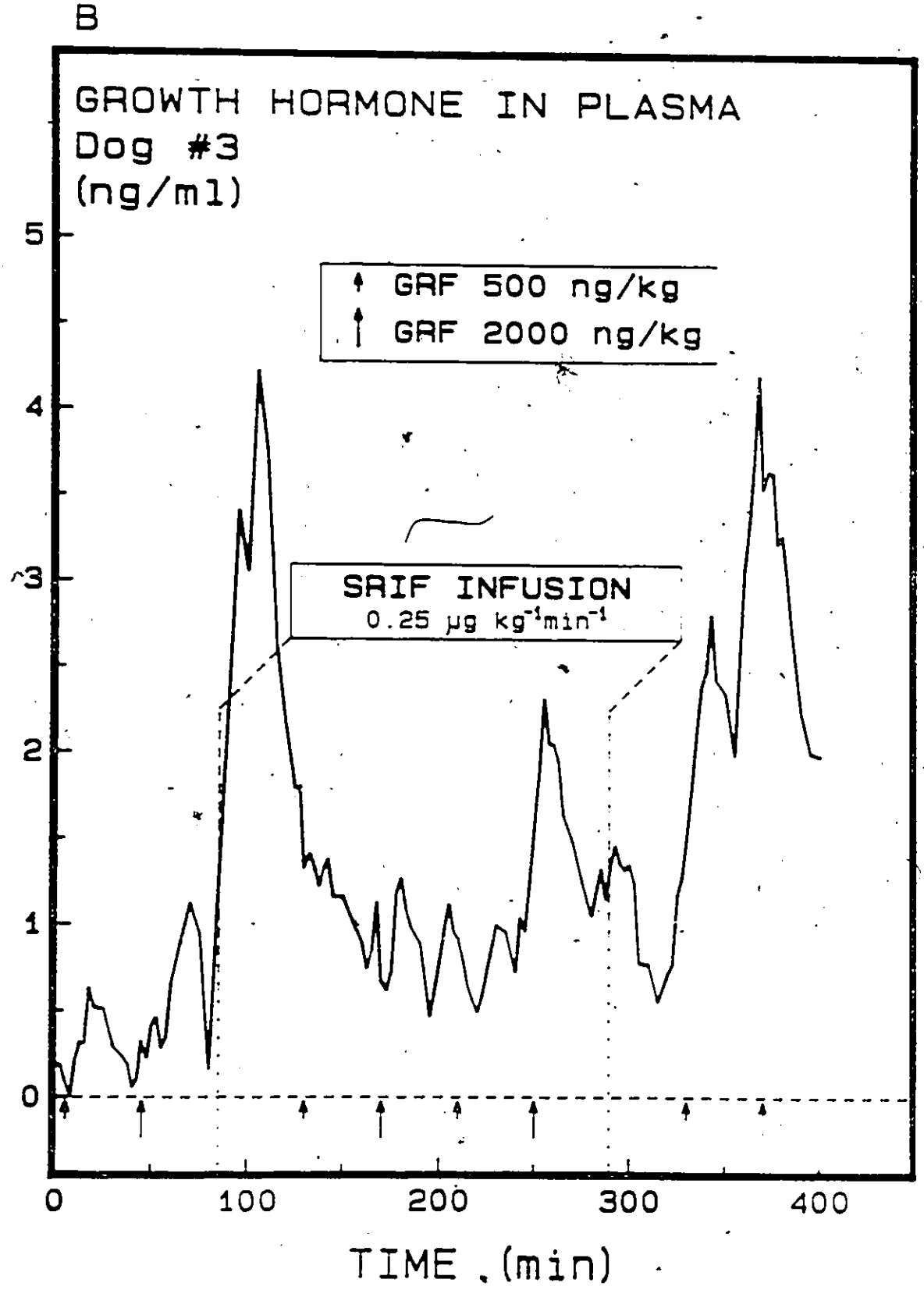

B
GROWTH HORMONE IN PLASMA
Dog #3
(ng/ml)
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
5
4
3
2
1
0
0
100
200
300
400
TIME (min)

Figure 2b) Growth hormone plasma concentration vs. time in experiment B4.

FIG 2b

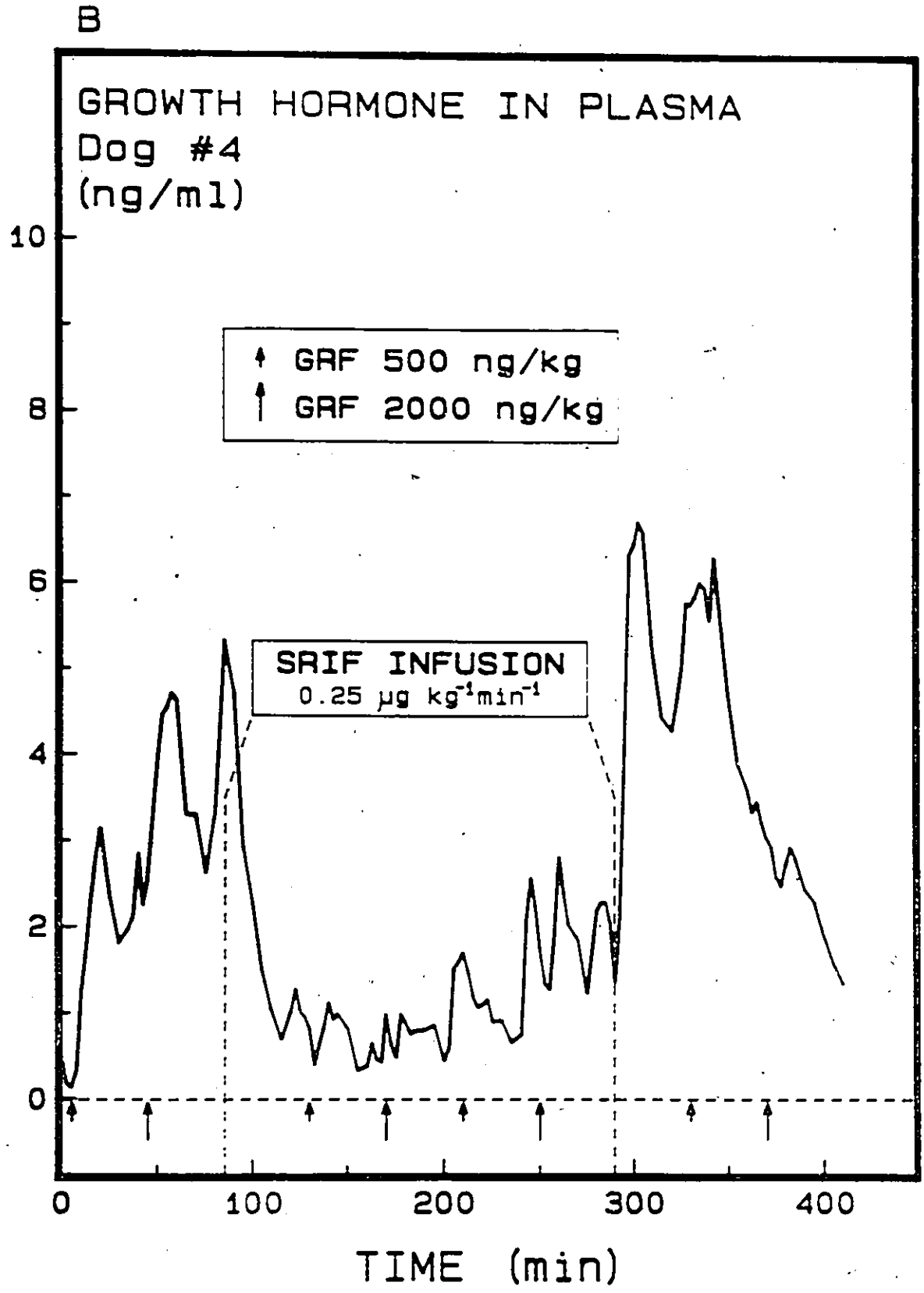

B
GROWTH HORMONE IN PLASMA
Dog #4
(ng/ml)
10
8
6
4
2
0
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
0
100
200
300
400
TIME (min)

Figure 2c) Growth hormone plasma concentration vs. time in experiment B5.

FIG 2c

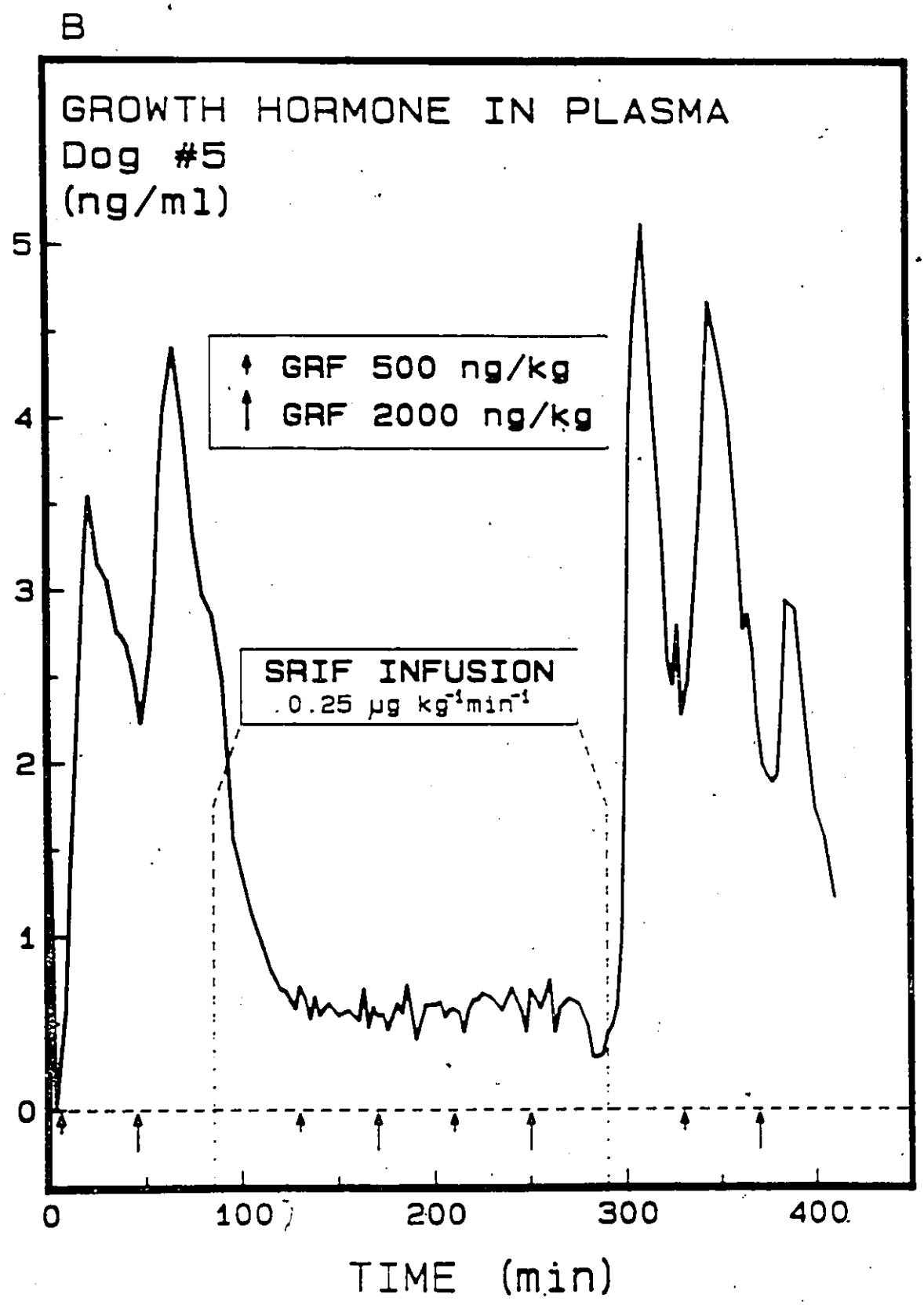
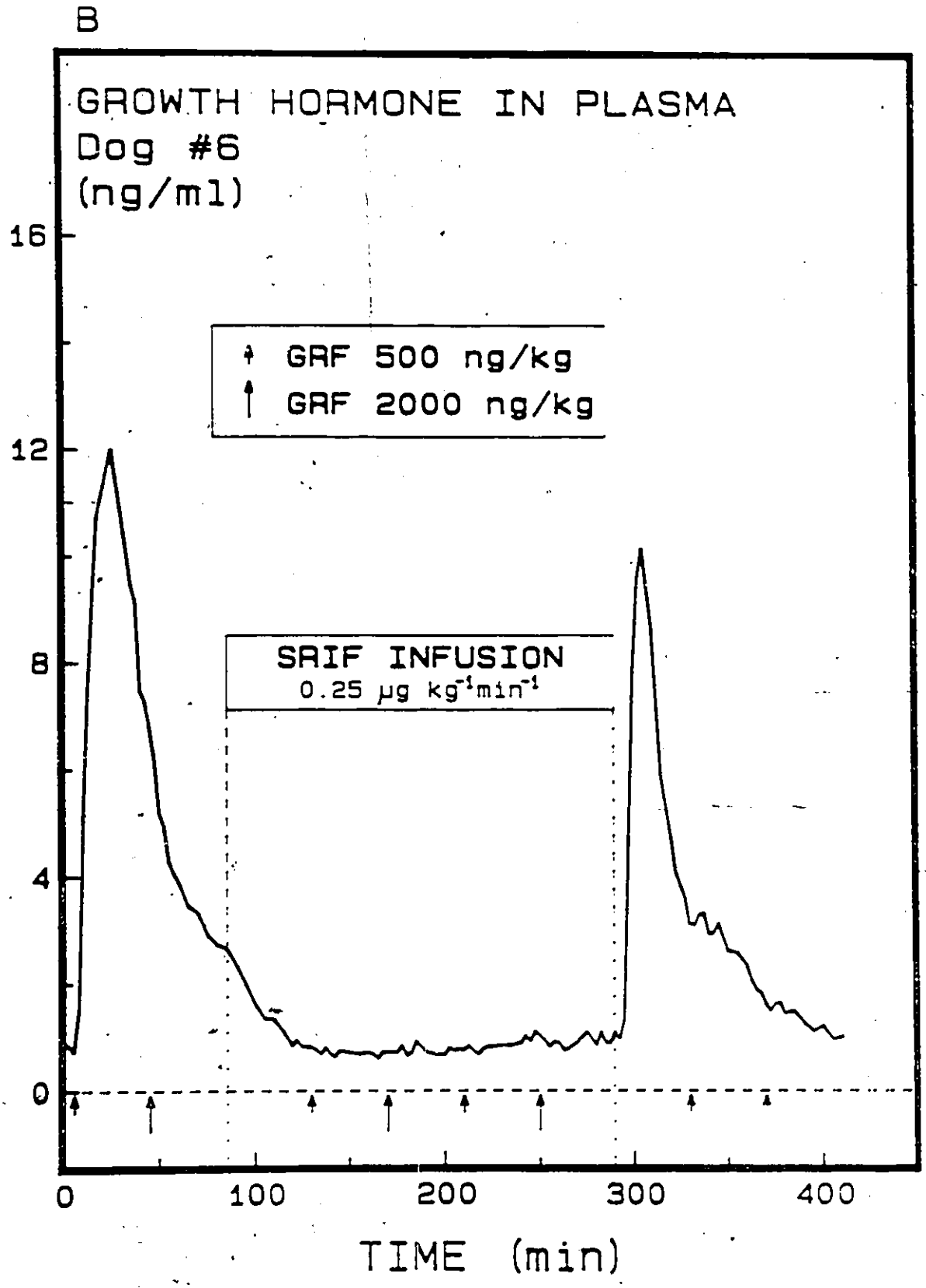

B
GROWTH HORMONE IN PLASMA
Dog #5
(ng/ml)
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
.0.25 µg kg⁻¹min⁻¹
5
4
3
2
1
0
0
100
200
300
400
TIME (min)

Figure 2d) Growth hormone plasma concentration vs. time in experiment B6.

FIG 2d


B
GROWTH HORMONE IN PLASMA
Dog #6
(ng/ml)
16
12
8
4
0
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
0
100
200
300
400
TIME (min)

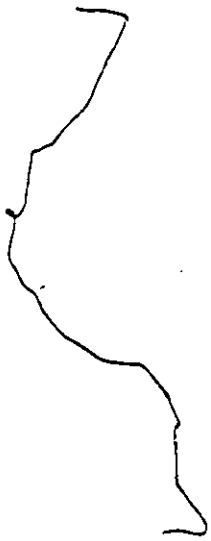


Figure 2e) Growth hormone secretion rates vs. time in experiment B3. Experiments B3-B6 contained both an initial and second control period, (pre-somatostatin and post-somatostatin, respectively), during which time 4 GRF injections were administered, (small and large arrows). Separating the 2 control periods was a 205 min somatostatin infusion with 4 GRF injections superimposed. Note that in all 4 experiments the cessation of the somatostatin infusion was followed by a growth hormone secretory overshoot, in 3 cases with no discernable lag (B4-B6), with a 15 min lag in B3.

FIG 2e

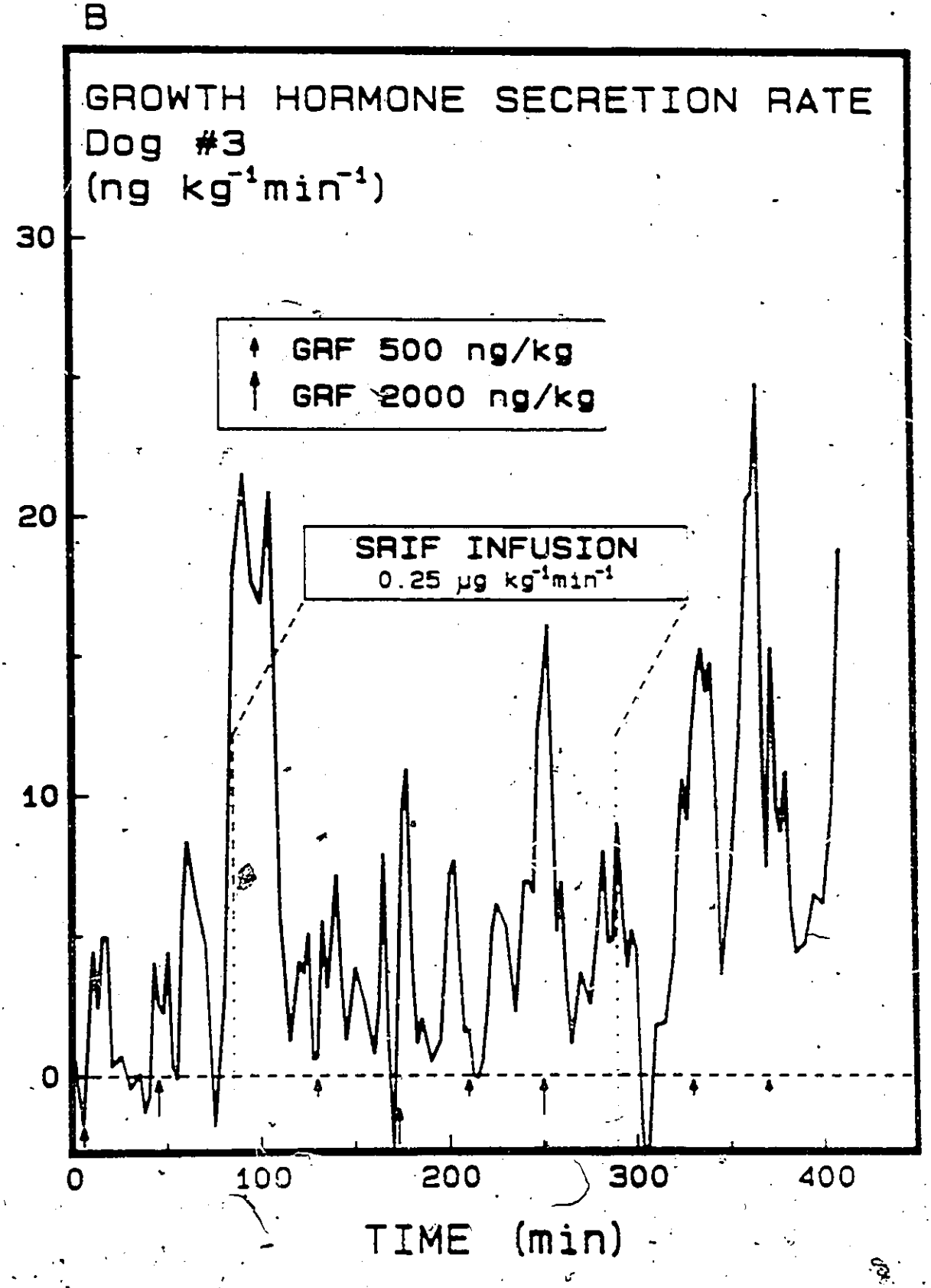

B
GROWTH HORMONE SECRETION RATE
Dog #3
(ng kg⁻¹min⁻¹)
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
30
20
10
0
0
100
200
300
400
TIME (min)

Figure 2f) Growth hormone secretion rates vs. time in experiment B4.

FIG 2f

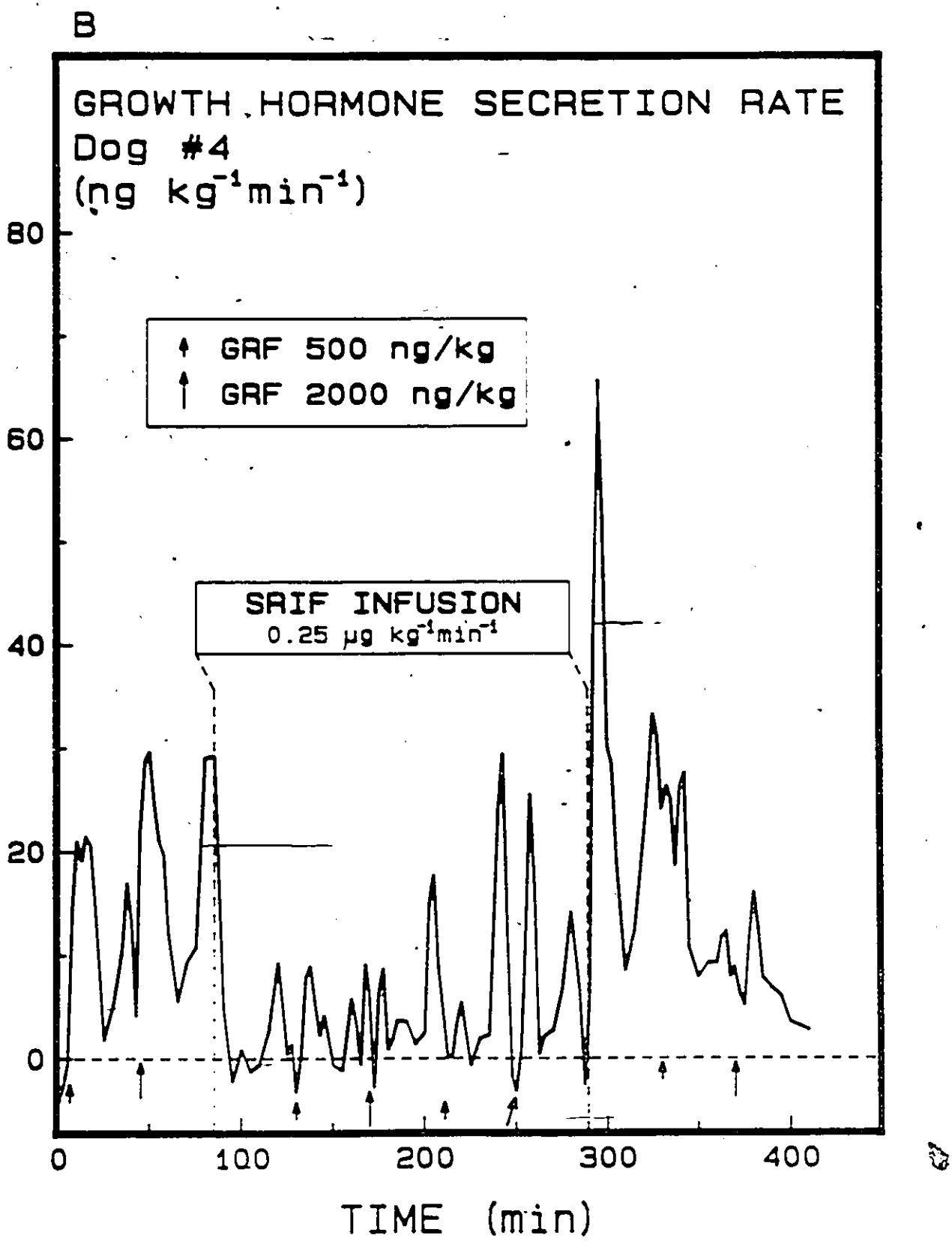

B
GROWTH HORMONE SECRETION RATE
Dog #4
(ng kg⁻¹min⁻¹)
80
60
40
20
0
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
0
100
200
300
400
TIME (min)

Figure 2g) Growth hormone secretion rates vs. time in experiment B5.

FIG 2g

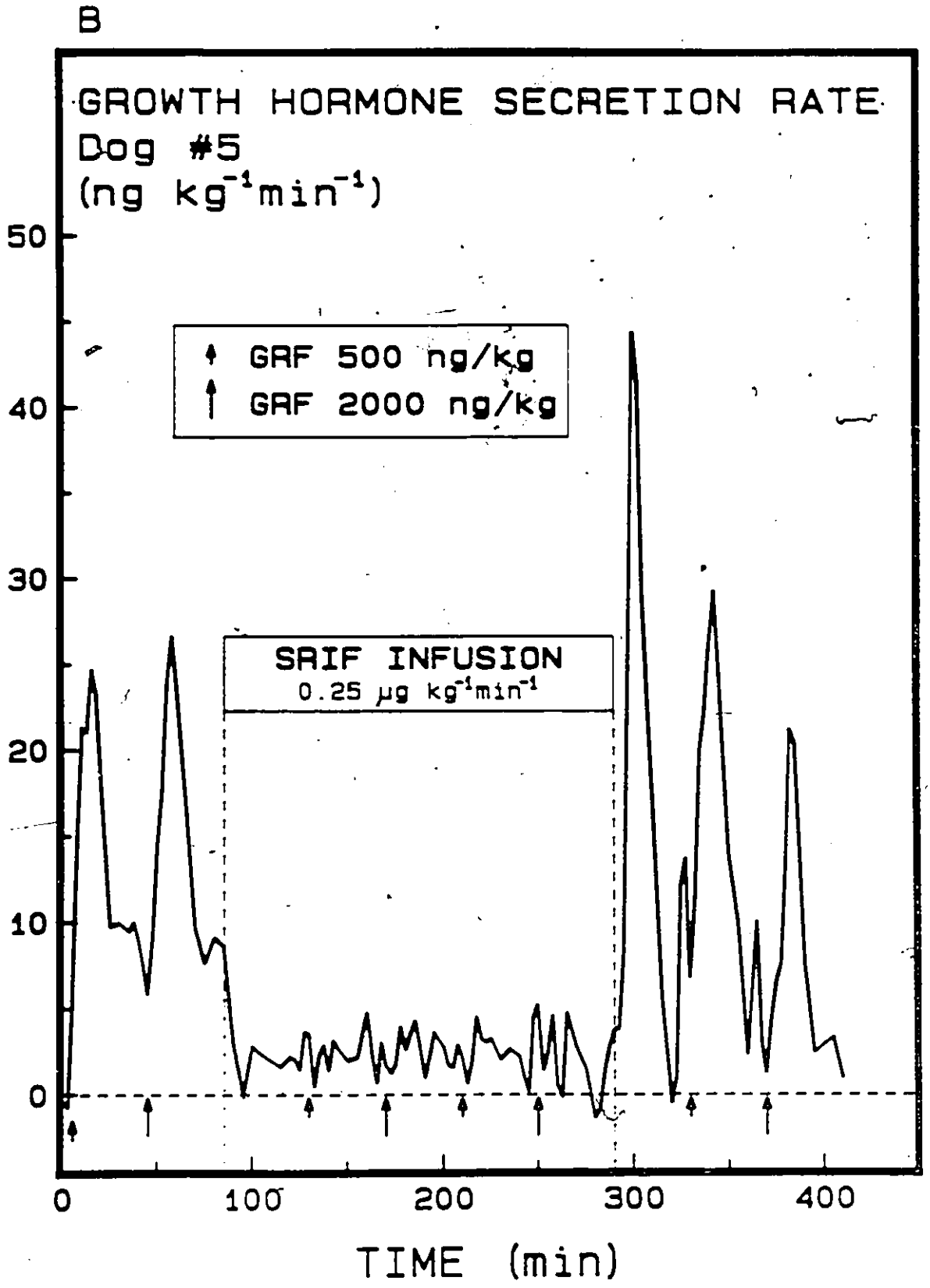

B
GROWTH HORMONE SECRETION RATE
Dog #5
(ng kg⁻¹min⁻¹)
50
40
30
20
10
0
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
0
100
200
300
400
TIME (min)

Figure 2h) Growth hormone secretion rates vs. time in experiment B6.

FIG 2h

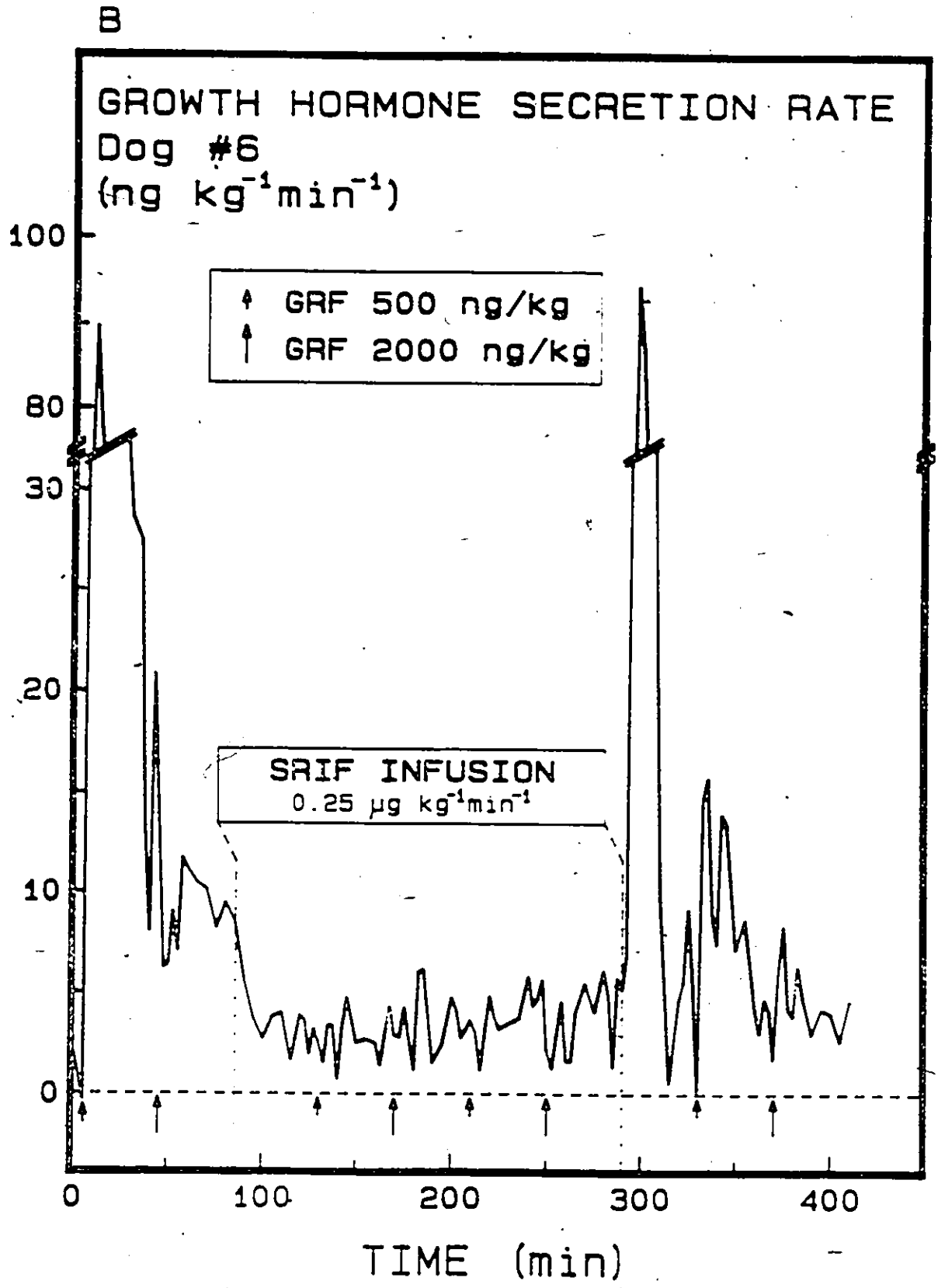

B
GROWTH HORMONE SECRETION RATE
Dog #6
(ng kg⁻¹min⁻¹)
GRF 500 ng/kg
GRF 2000 ng/kg
SRIF INFUSION
0.25 µg kg⁻¹min⁻¹
100
80
30
20
10
0
0
100
200
300
400
TIME (min)

Figure 3a) Growth hormone plasma concentration vs. time in B7.

FIG 3a

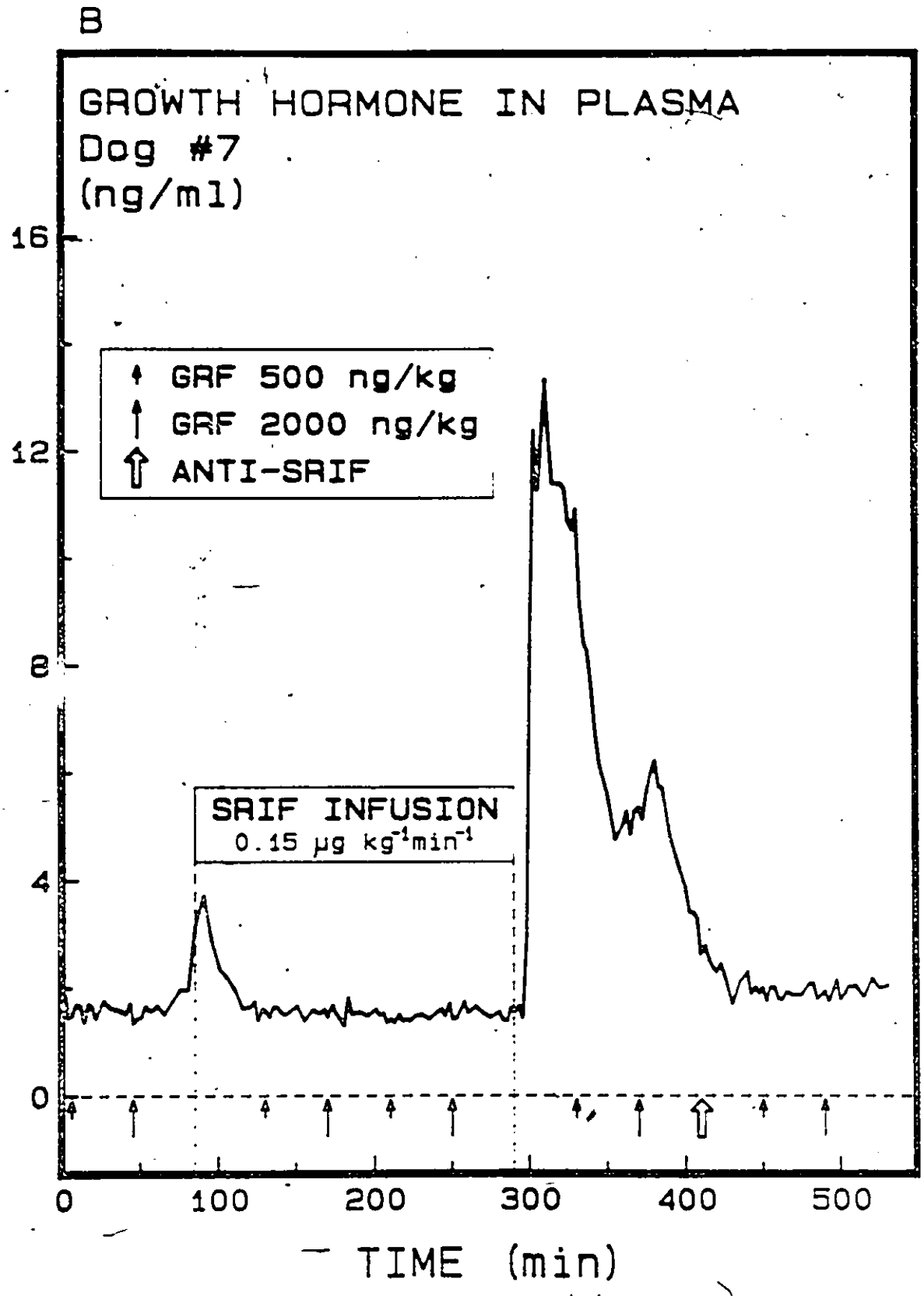

B
GROWTH HORMONE IN PLASMA
Dog #7
(ng/ml)
16
12
8
4
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 3b) Growth hormone plasma concentration vs. time in BS.

FIG 3b

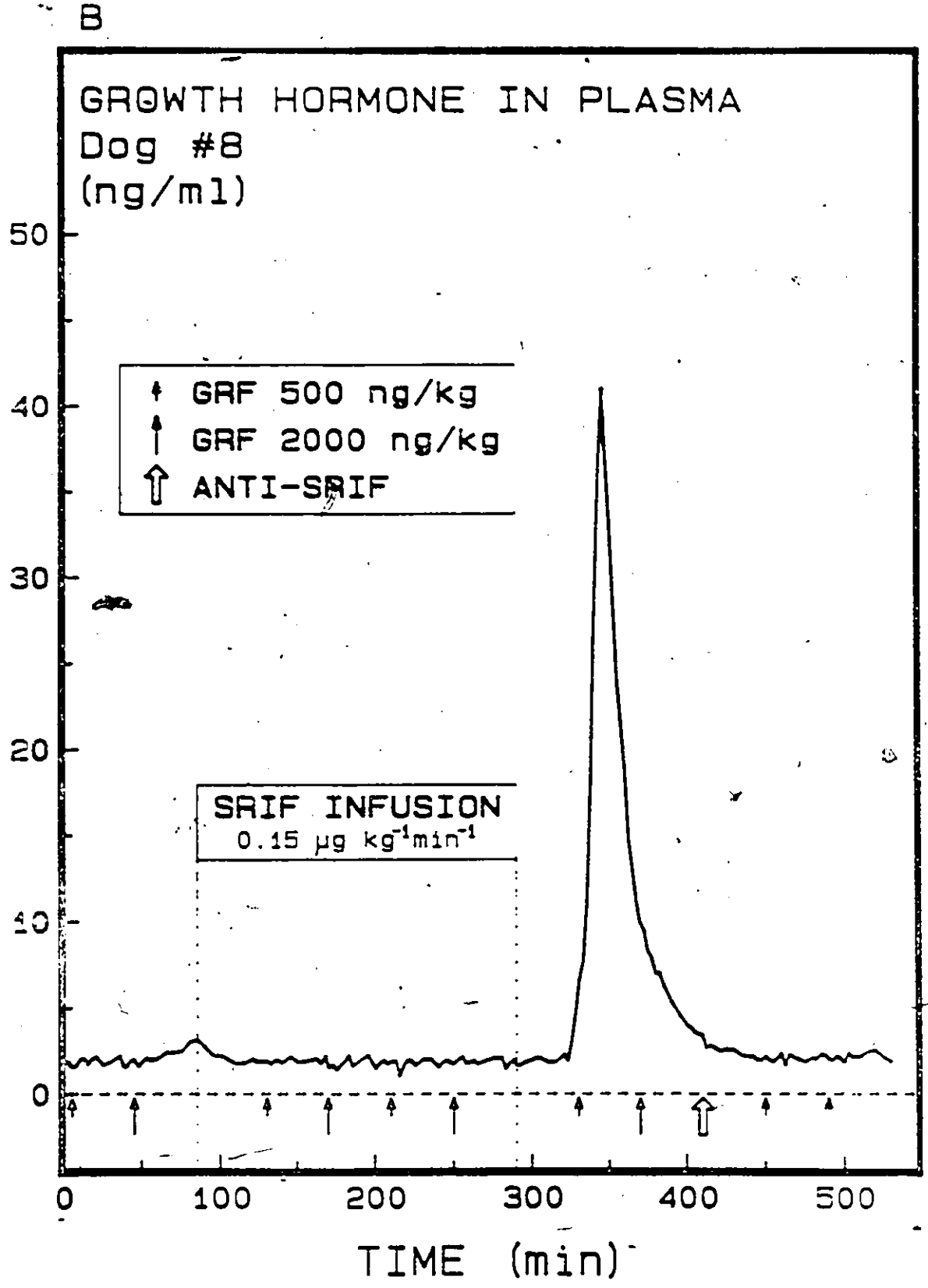

B
GROWTH HORMONE IN PLASMA
Dog #8
(ng/ml)
50
40
30
20
10
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 3c) Growth hormone secretion rates vs. time in B7. The somatostatin infusion in the 2 experiments was at a slightly lower rate than that in B1-B6; it was, however, previously shown to be effective in blocking all spontaneous growth hormone secretory bursts by Cowan et al. (1983). In B7 there was a post-somatostatin rebound present, with a 2.5 min lag. The monoclonal Ab to somatostatin soma #8 was administered at 410 min, but it was later shown that this Ab was less effective in vivo than soma #10 (see M series). There was no discernable response to the Ab in either dog.

FIG 3c

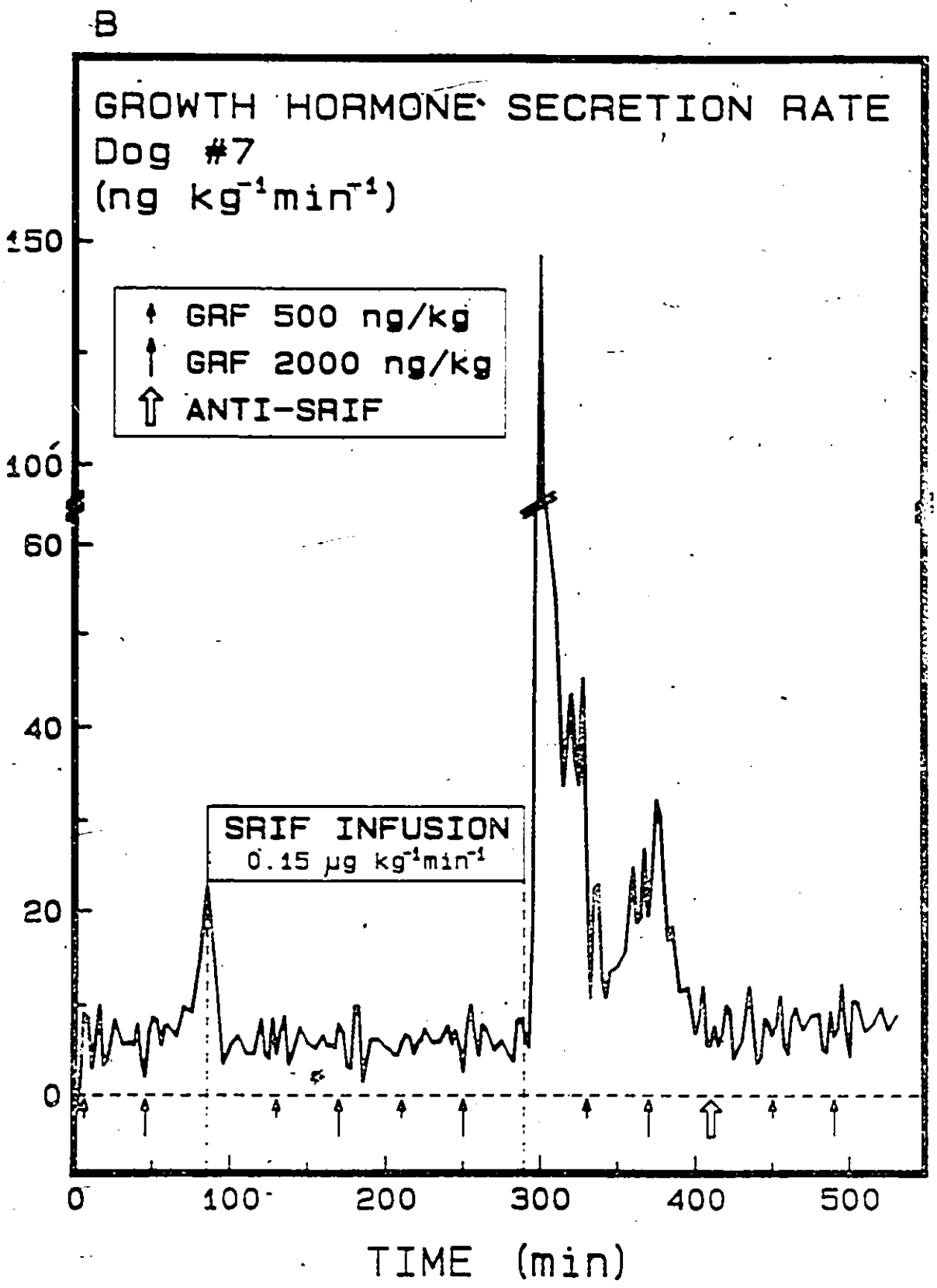

B
GROWTH HORMONE SECRETION RATE
Dog #7
(ng kg⁻¹min⁻¹)
150
100
60
40
20
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 3d) Growth hormone secretion rates vs. time in B8.

FIG 3d

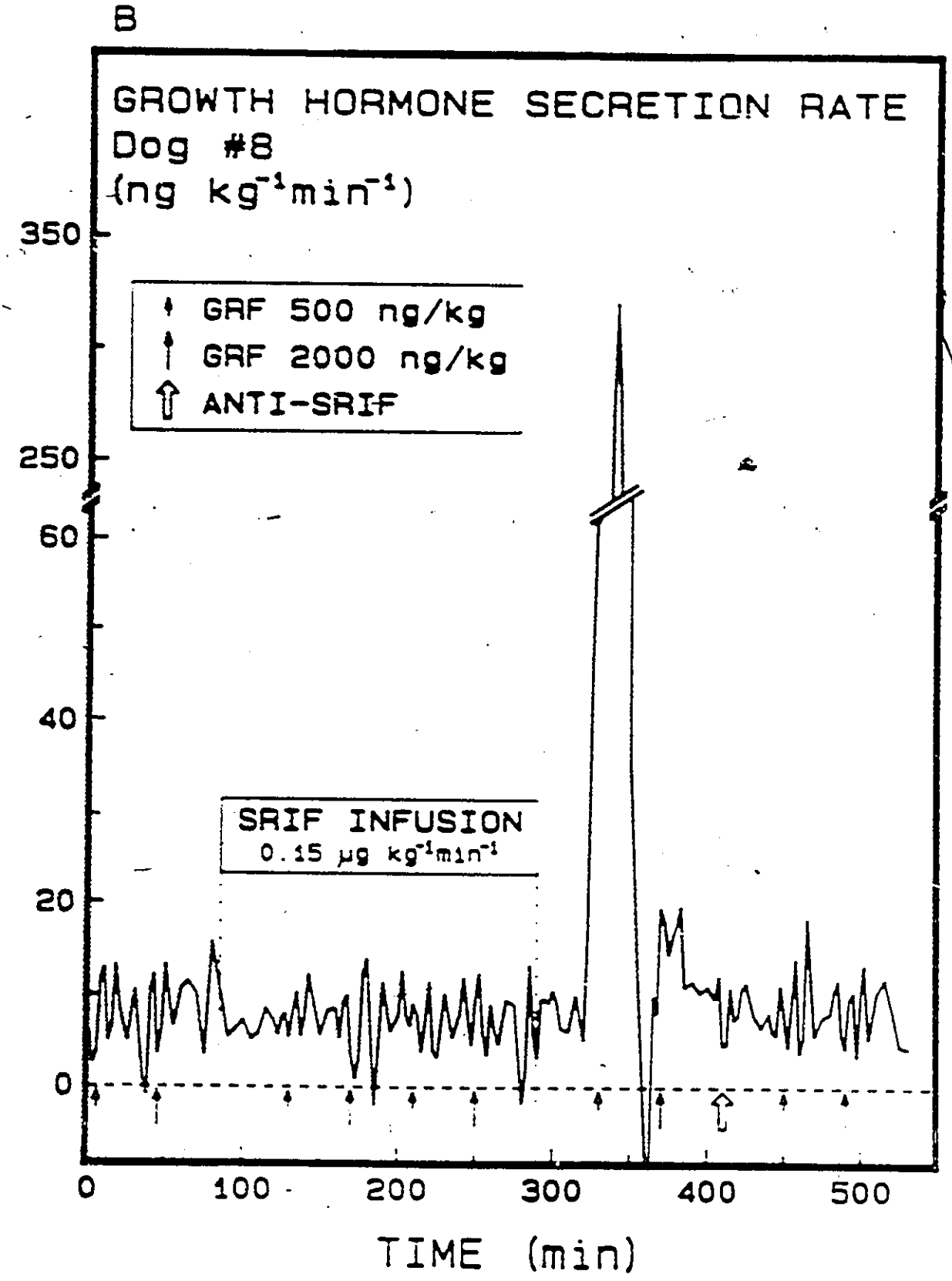

B
GROWTH HORMONE SECRETION RATE
Dog #8
(ng kg⁻¹min⁻¹)
350
250
60
40
20
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 4a) Growth hormone plasma concentration vs. time in Ml.

FIG 4a

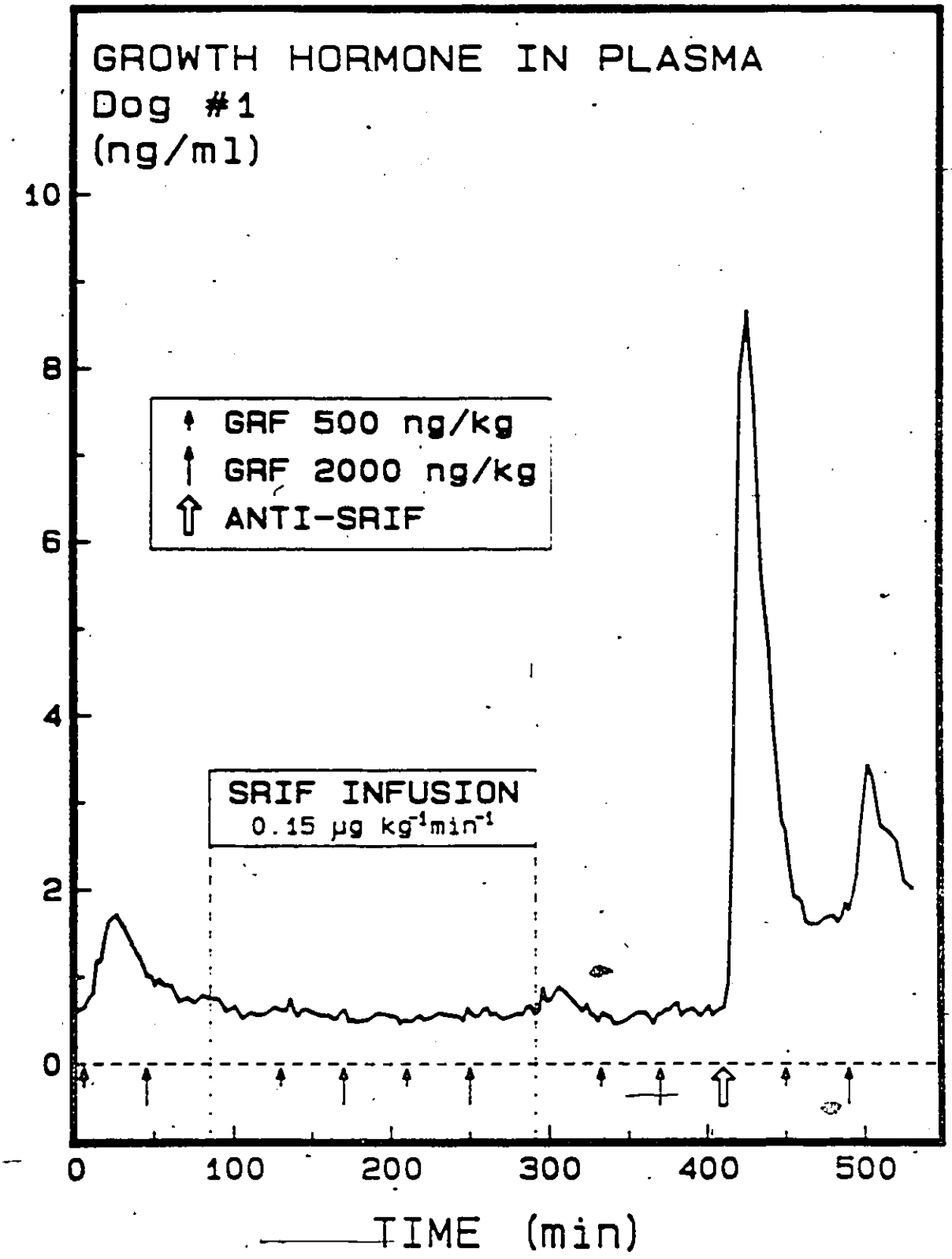

GROWTH HORMONE IN PLASMA
Dog #1
(ng/ml)
10
8
6
4
2
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 4b) Growth hormone plasma concentration vs. time in M2.

FIG 4b

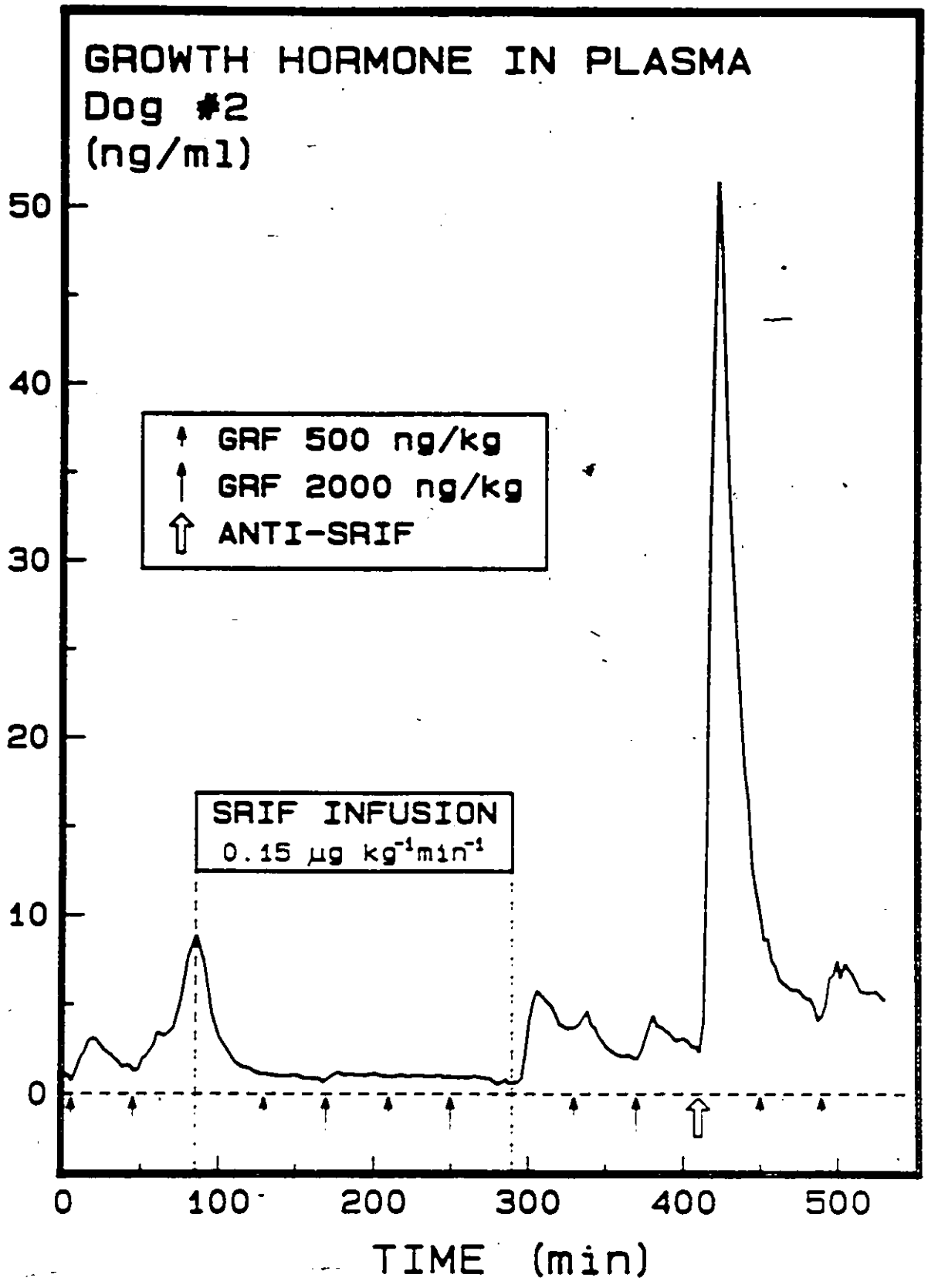

GROWTH HORMONE IN PLASMA
Dog #2
(ng/ml)
50
40
30
20
10
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 4c) Growth hormone plasma concentration vs. time in M3.

FIG 4c

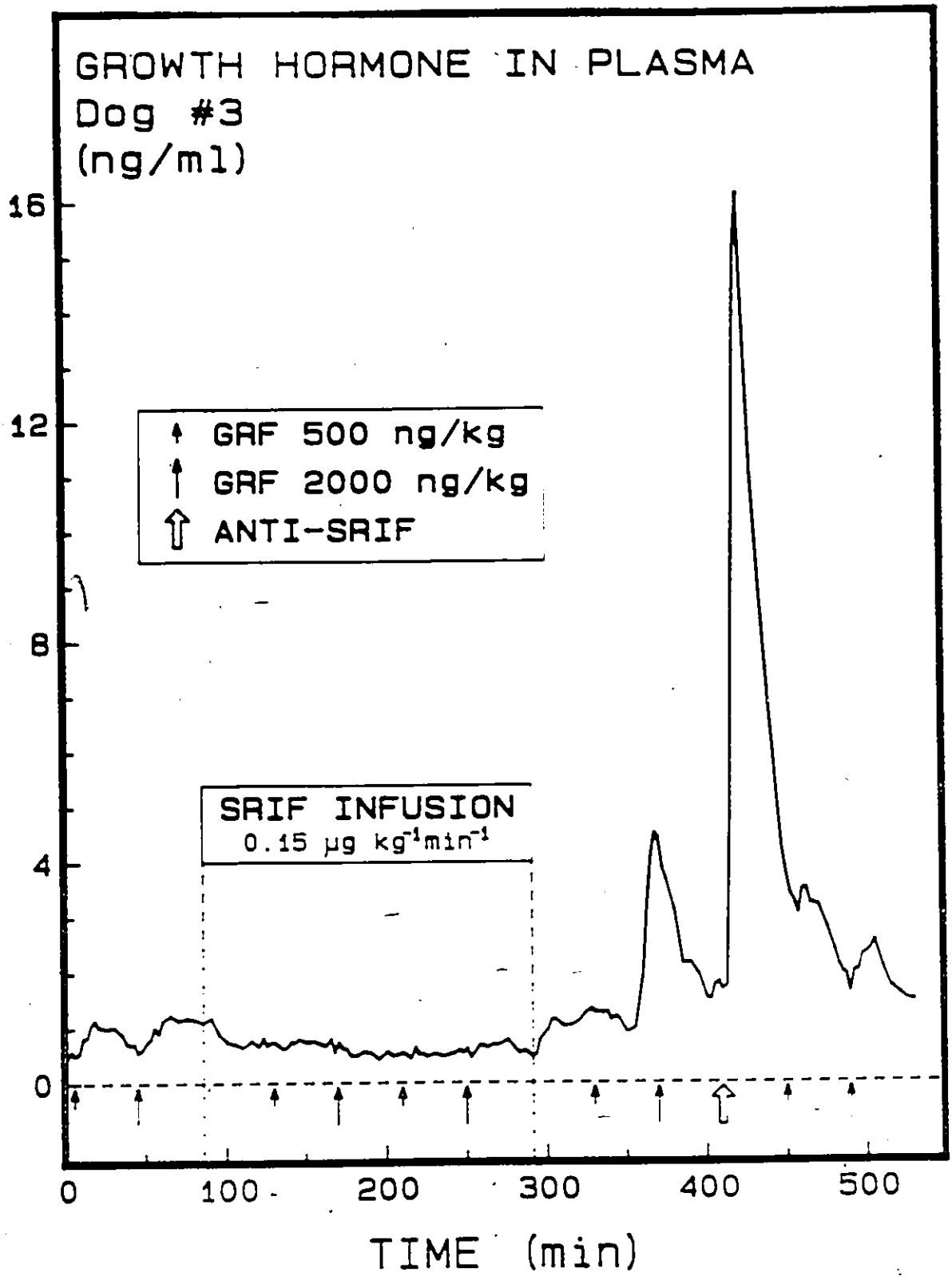

GROWTH HORMONE IN PLASMA
Dog #3
(ng/ml)
16
12
8
4
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 4d) Growth hormone plasma concentration vs. time in M5.

FIG 4d

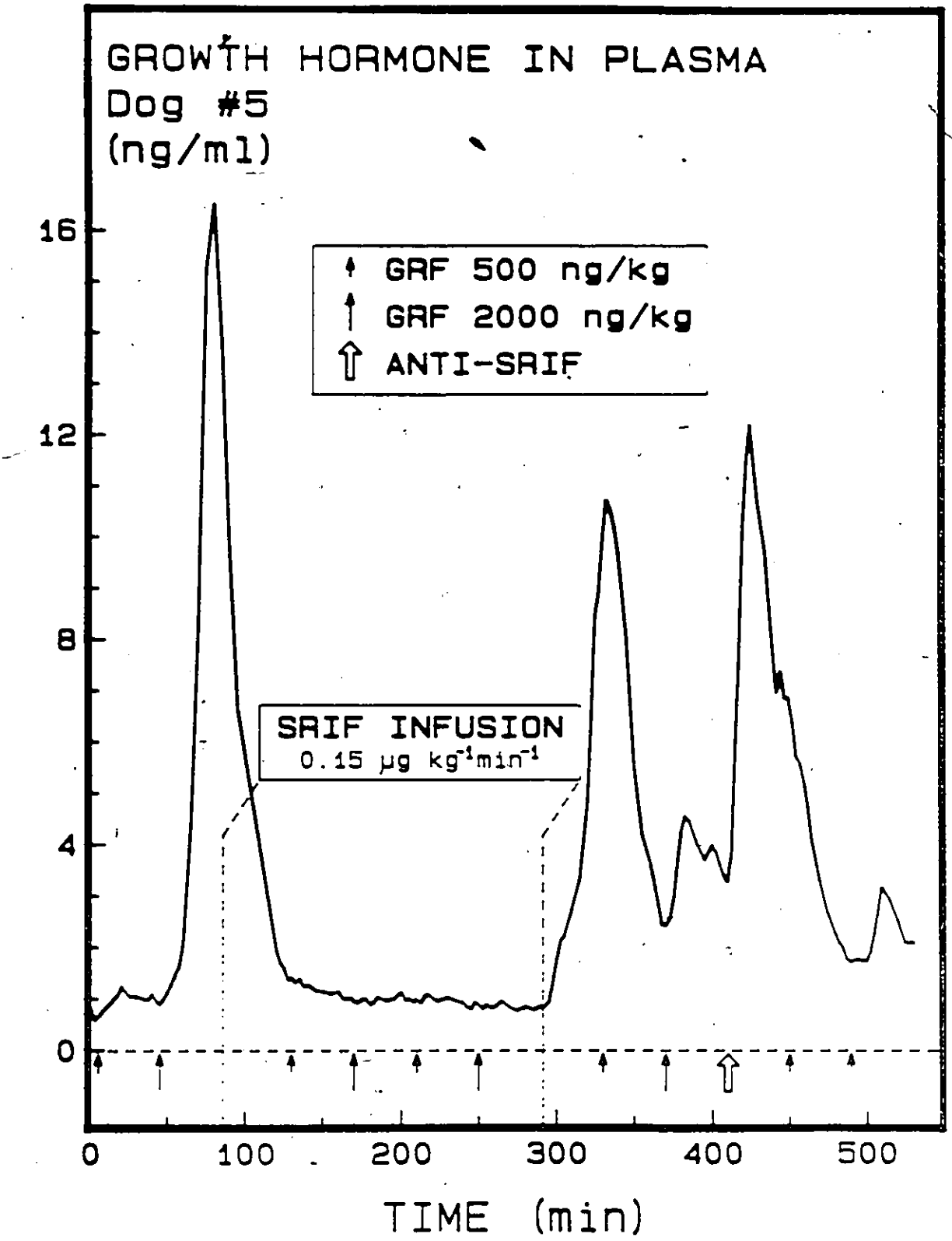

GROWTH HORMONE IN PLASMA
Dog #5
(ng/ml)
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
16
12
8
4
0
0
100
200
300
400
500
TIME (min)

Figure 4e) Growth hormone plasma concentration vs. time in M7.

FIG 4e

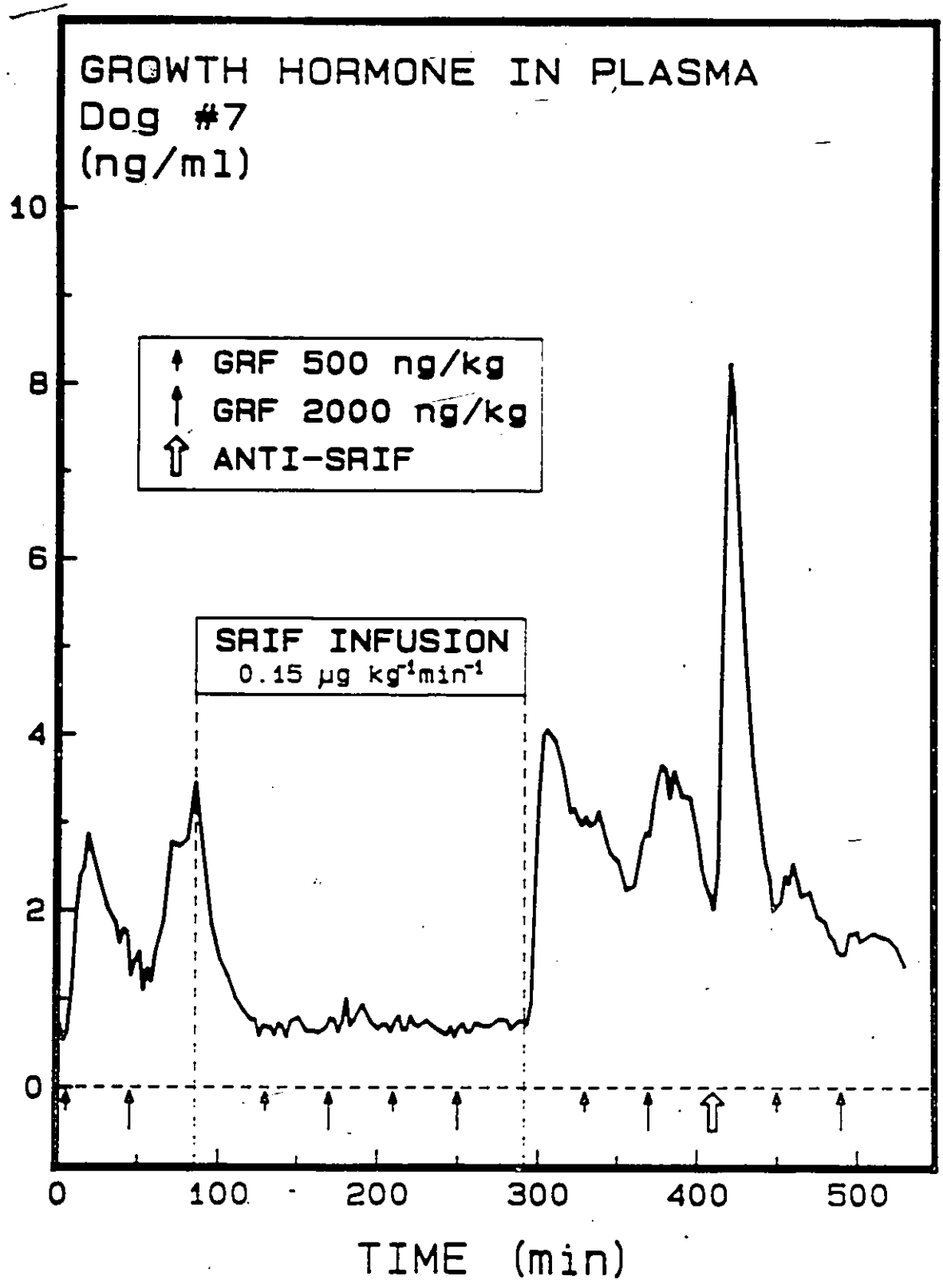

GROWTH HORMONE IN PLASMA
Dog #7
(ng/ml)
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
10
8
6
4
2
0
0
100
200
300
400
500
TIME (min)

Figure 4f) Growth hormone secretion rates vs. time in experiment M1. These experiments followed the same experimental protocol as the B series with 2 exceptions; the somatostatin infusion was at a slightly lower rate than that used in B1-B6 (0.15 $\mu g\ kg^{-1}\ min^{-1}$ vs. 0.25 $\mu g\ kg^{-1}\ min^{-1}$), and at 410 min, following the post-somatostatin period, an injection of monoclonal Ab to somatostatin soma #10 was administered. In these 5 experiments there was an immediate, large growth hormone secretory burst in response to the Ab, followed by an elevated baseline.

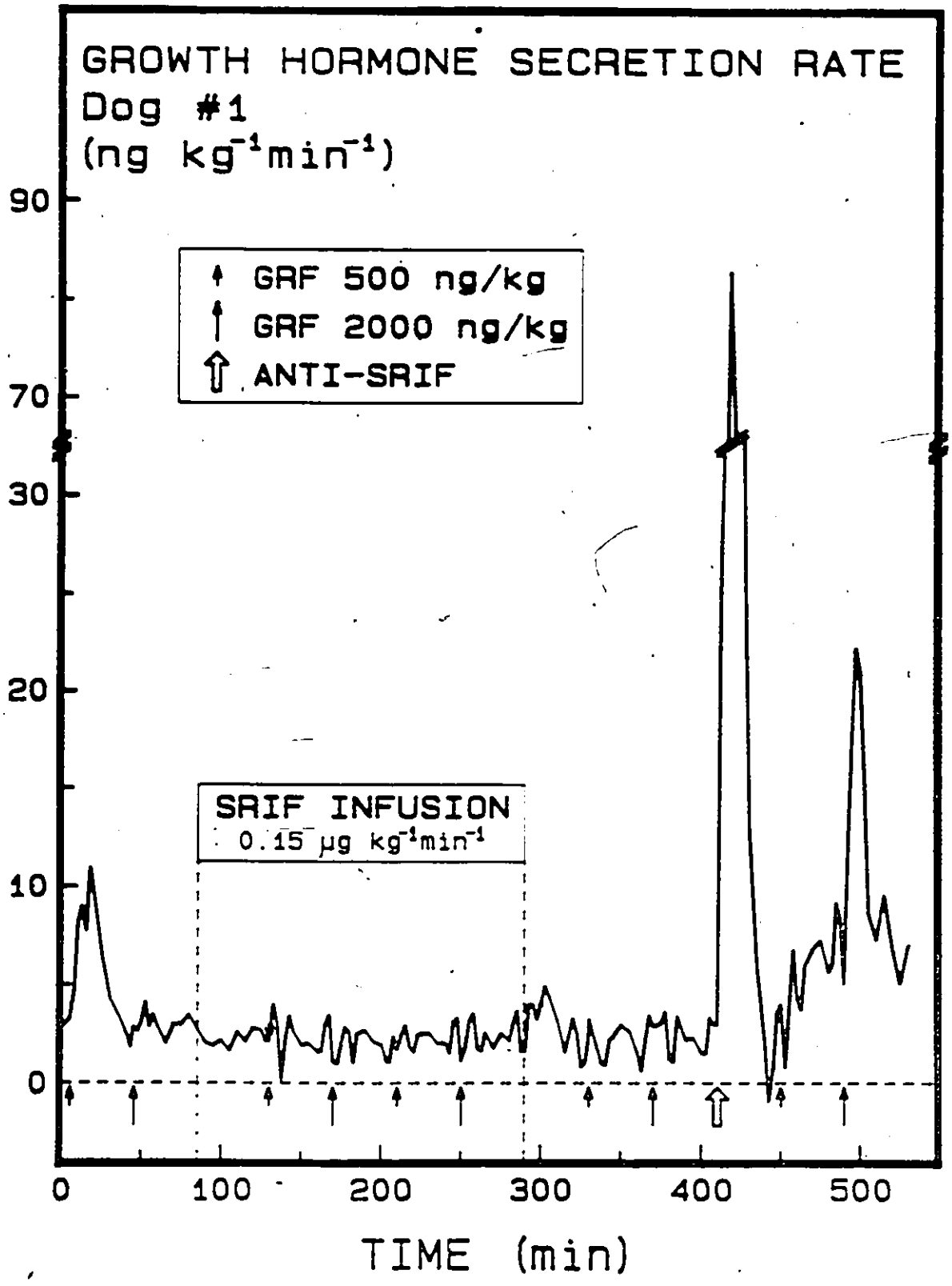

GROWTH HORMONE SECRETION RATE
Dog #1
(ng kg⁻¹min⁻¹)
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
90
70
30
20
10
0
0
100
200
300
400
500
TIME (min)

Figure 4g) Growth hormone secretion rates vs. time in experiment M2.

FIG 4g

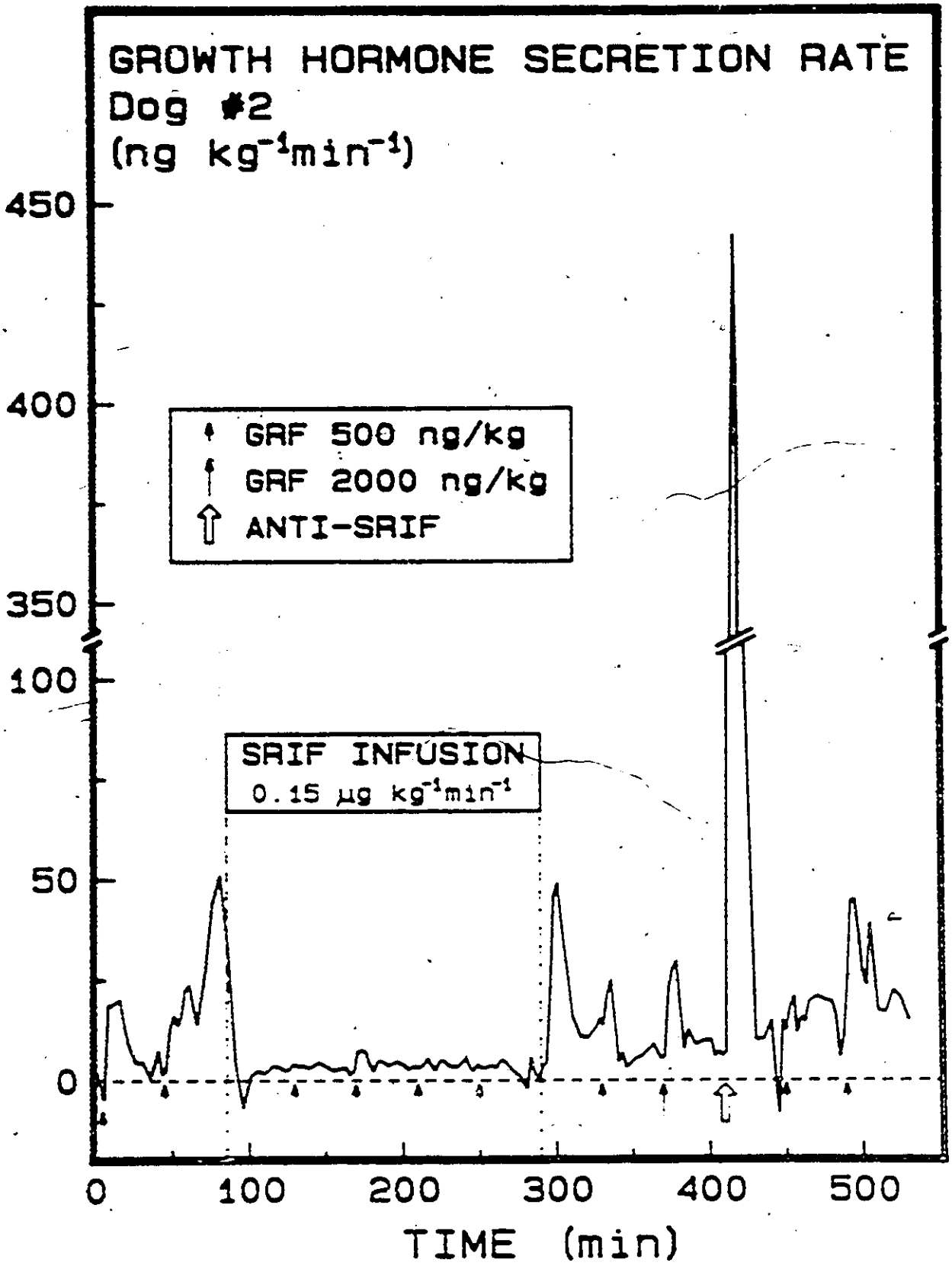

GROWTH HORMONE SECRETION RATE
Dog #2
(ng kg⁻¹min⁻¹)
450
400
350
100
50
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 4h) Growth hormone secretion rates vs. time in experiment M3.

FIG 4h

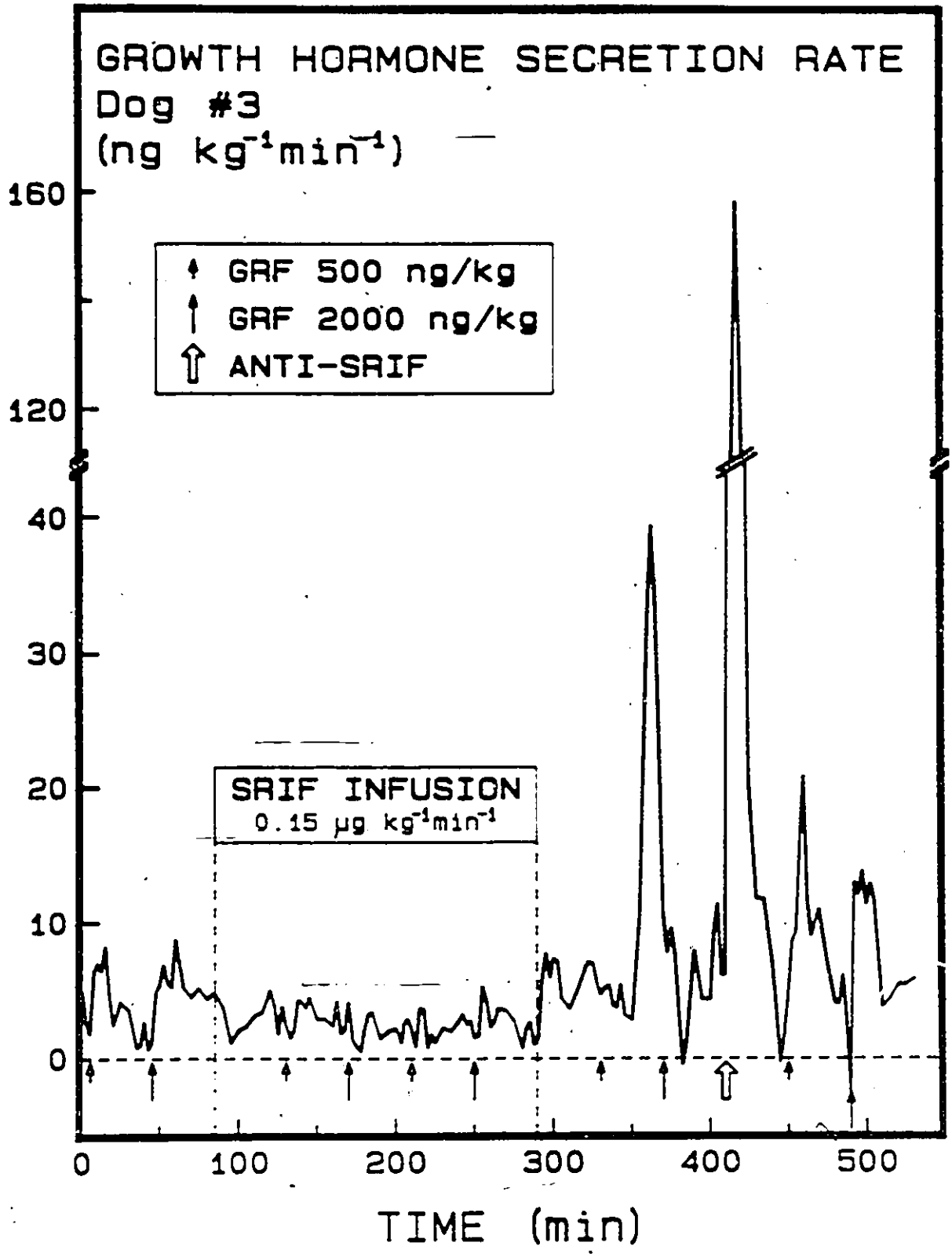

GROWTH HORMONE SECRETION RATE
Dog #3
(ng kg⁻¹min⁻¹)
160
120
40
30
20
10
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 4i) Growth hormone secretion rates vs. time in experiment M5.

FIG 41

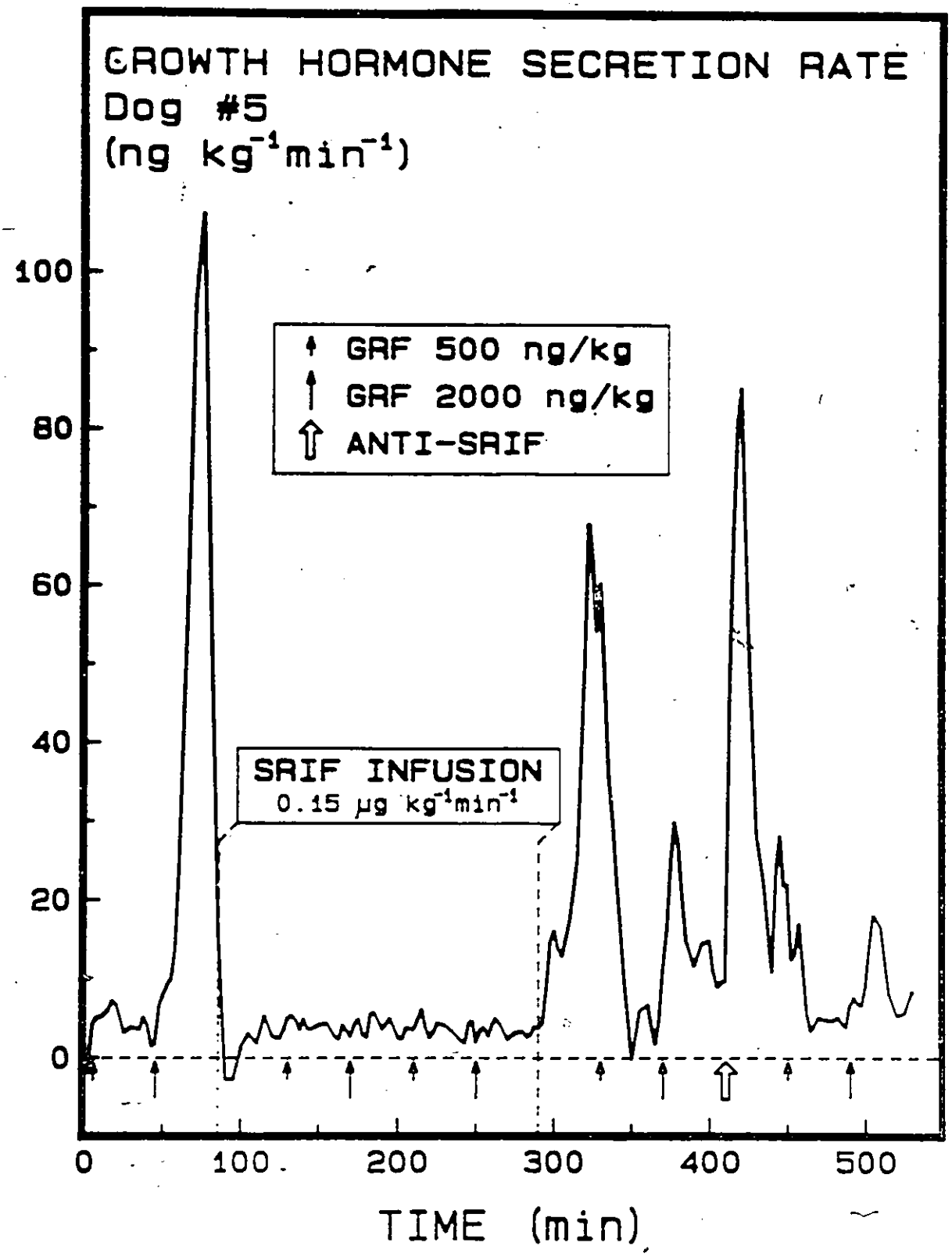

GROWTH HORMONE SECRETION RATE
Dog #5
(ng kg⁻¹min⁻¹)
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
100
80
60
40
20
0
0
100
200
300
400
500
TIME (min)

Figure 4j) Growth hormone secretion rates vs. time in experiment M7.

FIG 4j

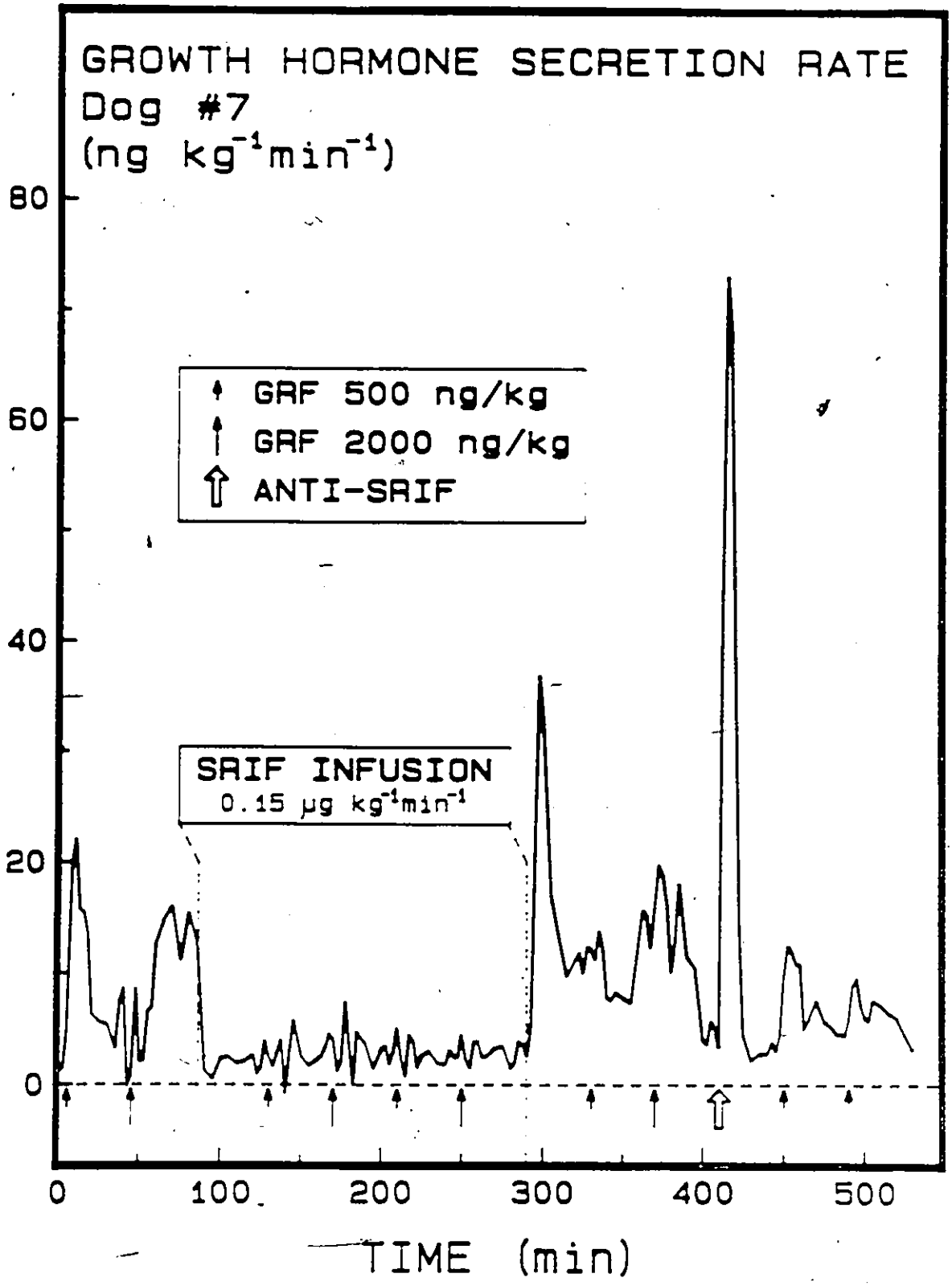

GROWTH HORMONE SECRETION RATE
Dog #7
(ng kg⁻¹min⁻¹)
80
60
40
20
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 5a) Growth hormone plasma concentration vs. time in experiment M4. There was not an immediate growth hormone secretory response to the monoclonal Ab to somatostatin in these 2 animals; there was, however, a slight elevation of baseline in M6 (1.5 fold increase) following the Ab administration (for explanation see Results).

FIG 5a

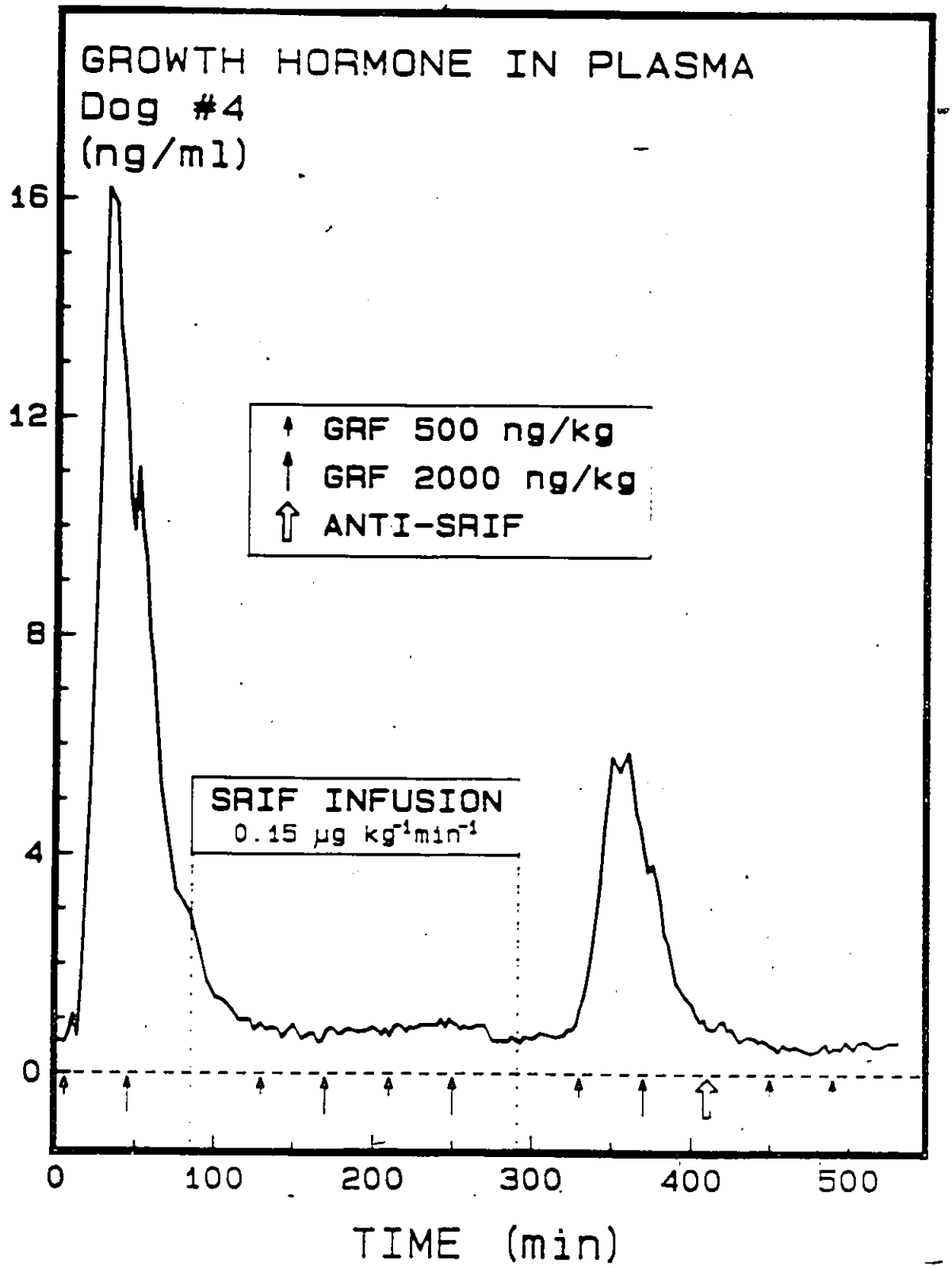

GROWTH HORMONE IN PLASMA
Dog #4
(ng/ml)
16
12
8
4
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 5b) Growth hormone plasma concentration vs. time in experiment M6.

FIG 5b

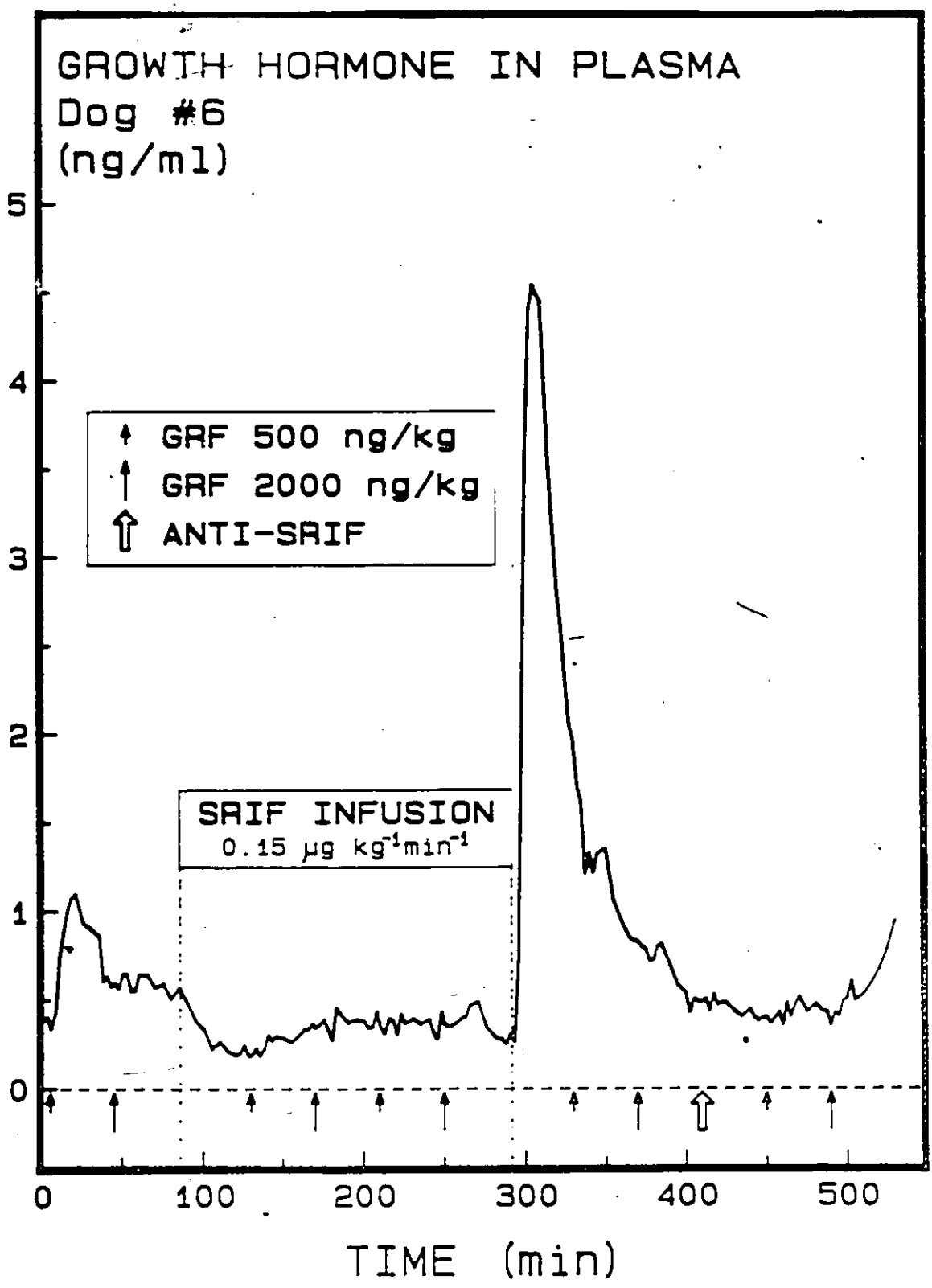

GROWTH HORMONE IN PLASMA
Dog #6
(ng/ml)
5
4
3
2
1
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 5c) Growth hormone secretion rates vs. time in experiment M4.

FIG 5c

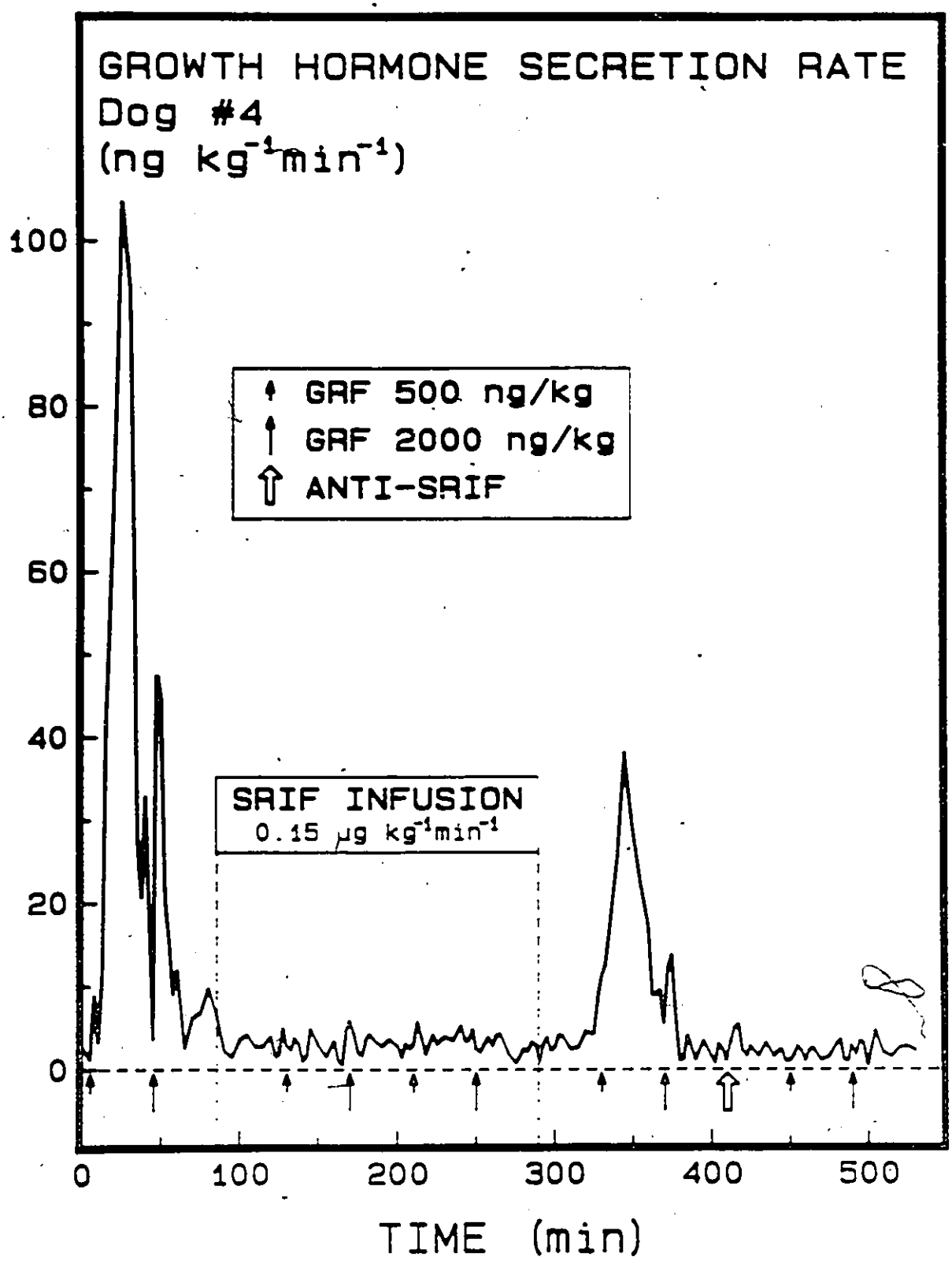

GROWTH HORMONE SECRETION RATE
Dog #4
(ng kg⁻¹min⁻¹)
100
80
60
40
20
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

Figure 5d) Growth hormone secretion rates vs. time in experiment M6.

FIG 5d

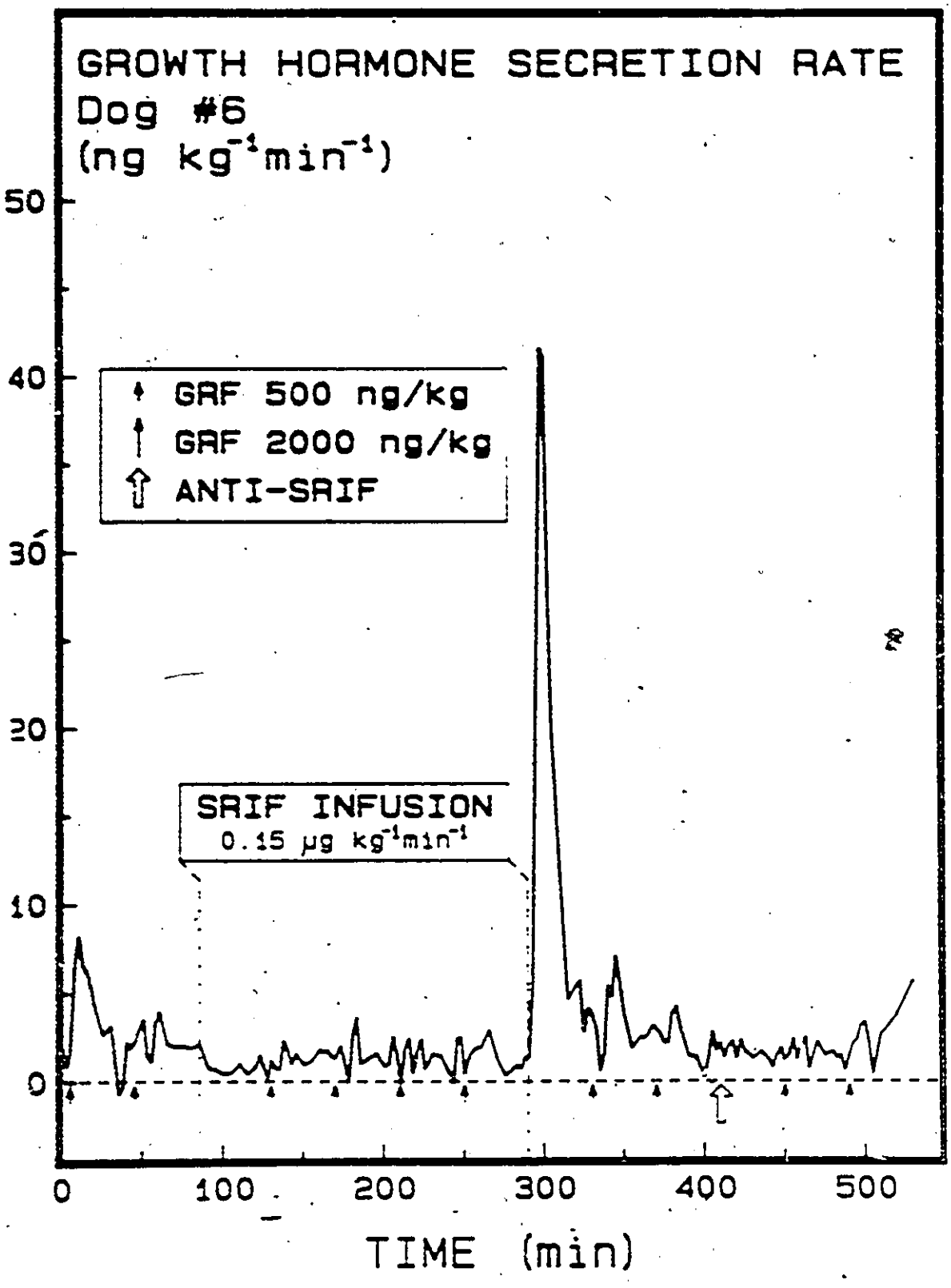

GROWTH HORMONE SECRETION RATE
Dog #6
(ng kg⁻¹min⁻¹)
50
40
30
20
10
0
GRF 500 ng/kg
GRF 2000 ng/kg
ANTI-SRIF
SRIF INFUSION
0.15 µg kg⁻¹min⁻¹
0
100
200
300
400
500
TIME (min)

TABLE 1: Summary of Animals, Exogenous Somatostatin and GRF, and Number of Samples; B Series

DOG #	EXP. #	WEIGHT (kg)	SRIF INFUSION		GRF INJECTIONS		#GH SAMPLES	#CORTISOL SAMPLES
			$\mu g\ kg^{-1}\ min^{-1}$	duration(min)	ng/kg	#		
B 1	B 1	18.5	0.25	240	125 & 500	6	100	6
B 1	B 2	19.2	0.25	240	125 & 500	6	100	6
B 2	B 3	12.6	0.25	205	500 & 2000	8	127	7
B 2	B 4	12.2	0.25	205	500 & 2000	8	127	7
B 3	B 5	17.4	0.25	205	500 & 2000	8	127	7
B 3	* B 6	17.5	0.25	205	500 & 2000	8	127	7
B 4	** B 7	14.6	0.15	205	500 & 2000	10	166	15
B 5	** B 8	16.0	0.15	205	500 & 2000	10	166	15

* The GRF peptide 1-40 was used, in place of hpGRF (1-44).

** In B7 and B8 a lower rate of somatostatin infusion was used (0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$), which has been shown by Cowan et al. (1984) to be effective in blocking spontaneous bursts of growth hormone release in dogs. The monoclonal Ab to somatostatin soma #8 was used in these two animals but was later shown to have a lower affinity in vitro for somatostatin and to be less effective in vivo than soma #10.

TABLE II: Summary of Animals, Experimental Design and Exogenous Material; M Series

DOG #	WEIGHT (kg)	Ab INJECTION (λ/kg)	205 min Somatostatin Infusion ($0.15\ \mu g^{-1}\ kg^{-1}\ min$)	hpGRF (1-44) 500 ng/kg	hpGRF (1-44) 2000 ng/kg	# GH SAMPLES	# GLUCOCORTICOID SAMPLES
M 1	12.70	23.6		5	5	166	15
M 2	14.25	21.05		5	5	166	15
M 3	16.25	12.31		5	** 5	166	15
M 4	23.50	*20.00		5	5	166	15
M 5	15.50	20.00		5	5	166	15
M 6	14.20	20.00		5	5	166	15
M 7	16.40	30.00		5	5	166	15

* The exact quantity of Ab administered was not known, through an error.

** The last large injection was 1500 ng/kg.

TABLE III: Summary of Experimental Designs

EXPERIMENT #	PRE-SOMATOSTATIN (control #1)	SOMATOSTATIN INFUSION	POST-SOMATOSTATIN (control #2)	POST-Ab
B 1 & B 2	no	yes	yes	no
B 3 - B 6	yes	yes	yes	no
B 7 & B 8	yes	yes	yes	*
M 1 - 7	yes	yes	yes	yes

* In B7 and B8 the monoclonal Ab to somatostatin soma *8 was used; however, it was later shown to have a lower affinity in vitro for somatostatin and to be less effective in vivo than soma #10. Because of the lack of response to the Ab at the quantity used, these animals were included in the B series.

TABLE IV: Frequency of Response to Injections of hpGRF (1-44); Individual Experiments (# of responses/# of possible responses)

EXP. #	C #1	SRIF INFUSED	C #2	POST-Ab
B 1	----	0/3	2/2	----
2	----	0/4	0/1	----
3	2/2	0/3	0/0	----
4	1/1	1/3	0/1	----
5	2/2	0/4	2/2	----
6	1/2	0/4	2/2	----
7	0/2	0/4	2/2	0/2
8	0/2	0/4	0/1	0/2
M 1	1/2	0/4	0/2	1/2
2	2/2	0/4	2/2	0/1
3	0/2	0/4	0/1	2/2
4	2/2	0/4	1/1	*0/2
5	1/2	0/4	0/0	1/1
6	1/2	0/4	1/2	*0/2
7	1/2	0/4	0/0	1/1
TOTAL	14/25	1/57	12/19	*5/11
% RESPONSE	56.0%	1.8%	63.2%	71.4%

* M4 and M6 did not show an immediate response to the monoclonal Ab to somatostatin, although M6 did show a slight elevation of basal growth hormone concentration. If these 2 post-Ab periods are not included in the calculations it results in a 71.4% response rate for GRF injections which follow an Ab-induced growth hormone burst.
Not all GRF injections were possible to respond to, due to spontaneously occurring bursts and long responses to previous events. The percentage of responses is calculated based on the number of responses to possible responses.

TABLE V: Summary of Frequency of Response to Injections of hpGRF (1-44)

(# of responses/# of possible responses)

SERIES (n)	CONTROL #1	SOMATOSTATIN INFUSION	CONTROL #2	POST-Ab
B (n=8)	6/11	1/29	8/11	----
M (n=7)	8/14	0/28	4/8	*5/7
Total (n=15)	14/25	1/57	12/19	5/7
% Response	56%	1.8%	63.2%	*71.4%

* These 5 responses to GRF injection all occurred in the 5 experiments in which the Ab evoked a growth hormone burst immediately. Of the 10 GRF injections following those bursts, only 7 could have evoked visible responses, since 3 were obscured by other events (see text). Hence 5/7 is the appropriate ratio here if one considers only responses to GRF after Ab-induced growth hormone bursts.

TABLE VI: Characteristics of Growth Hormone Secretory Bursts in Response to hpGRF (1-44) during Control, Somatostatin Infused and Post-Ab Periods

	CONTROL #1	SOMATOSTATIN INFUSION	CONTROL #2	POST-Ab
lag (min)	1.4	0	1.6	0.7
*Total Extra Secretion (ng/kg)	560.23 ± 170.79	150.3	168.37 ± 36.13	127.4 ± 29.3
Duration (min)	31.43 ± 2.76	12.5	20.00 ± 2.67	21.00 ± 2.8
Number	n=14	n=1	n=12	n=5

* Total extra secretion due to a secretory burst was calculated from the integrated peak area minus basal secretion.

TABLE VII: Frequency of Spontaneous Growth Hormone Secretory Bursts(all 15 expts)

BURSTS	CONTROL PERIODS	SOMATOSTATIN INFUSION	POST-Ab
Number	17	5	2
frequency	1/2.85 hr.	1/10.22 hr.	* 1/3.65 hr.

* The Post-Ab frequency was calculated by subtracting the average time of the response to the Ab from this period.

TABLE VIII: Characteristics of Spontaneous Growth Hormone Secretory Bursts During Control, Somatostatin Infused and Post-Ab Periods

	C #1	SRIF INFUSED	C #2	SUCCESSFUL POST-Ab
Frequency (per hour)	1/2.63	1/10.22	1/3.00	1/5 hr.
Total Extra GH Secretion (ng/kg)	367.7 ± 79.2	156.9 ± 32.6	382.9 ± 50.5	273.5 ± 183.3
Duration (min)	28.2 ± 3.3	20.5 ± 3.9	37.8 ± 2.4	21.3 ± 3.8
Number	7	5	10	2

TABLE IX: Characteristics of the Post-Somatostatin Rebound in Growth Hormone

EXP. #	PEAK GH SECRETION Rate (ng $kg^{-1}min^{-1}$)	DURATION (min)	TOTAL EXTRA GH SECRETION (ng/kg)	LAG (min)
B 1	8.42	30	84.36	10
2	10.62	27.5	140.59	0
3	15.29	40	310.66	15
4	65.57	20	499.72	0
5	44.31	30	488.21	0
6	94.25	25	904.74	0
7	148.41	40	1656.77	2.5
8	------	---	---------	------
M 1	------	---	---------	------
2	49.65	25	457.78	0
3	------	---	---------	------
4	------	---	---------	------
5	67.79	60	1531.23	0
6	41.54	35	474.00	0
7	36.83	25	367.20	0
Total ($\bar{x}$)	52.97 ± 12.38	32.5 ± 3.4	628.7 ± 157.9	2.50 ± 1.54

In all experiments with the higher rate of somatostatin infusion, (B1-B6) a post-somatostatin rebound occurred, with no discernable lag (2.5 min) between the cessation of the somatostatin infusion and the initiation of the secretory overshoot in 8 out of 11 rebounds.

TABLE X: Characteristics of the Immediate Growth Hormone Secretory Response to Somatostatin Ab Injection (soma #10)

EXP. #	PEAK GH SECRETION RATE (ng kg^{-1} min^{-1})	DURATION (min)	TOTAL EXTRA GH SECRETION (ng/kg)	LAG (min)
M 1	82.67	32.5	903.76	0
2	445.92	20	4389.15	0
3	158.37	35	1397.56	0
** 4	------	----	-------	---
5	85.14	55	1407.97	0
** 6	------	----	-------	---
7	73.02	20	572.71	0
Total ($\bar{x}$ ± S.E.M.)	169.02 ± 70.89	32.5 ± 6.4	*1734.2 ± 682.2	0

* The large secretory response to the Ab was due to the amplitude (peak secretion rate), with duration comparable to spontaneously occurring bursts during the control periods.

** M4 and M6 failed to show an immediate response to the Ab injection, although M6 did have a slightly elevated basal growth hormone concentration (1.5 fold increase) in the post-Ab period.

TABLE XI: Basal Plasma cGrowth Hormone Concentration (ng/kg)

EXP. #	CONTROL #1	SOMATOSTATIN INFUSION	CONTROL #2	POST-Ab
B1	*	.26	---	
B2	*	.20	---	
B3	.12	.97	---	
B4	---	.76	---	
B5	---	.57	---	
B6	.81	.83	---	
B7	1.58	1.51	1.93	
B8	1.86	1.84	1.92	
M1	.74	.57	.60	1.68
M2	.92	.91	---	6.03
M3	.87	.62	1.09	1.48
M4	.58	.78	.66	.52
M5	.92	.93	---	1.91
M6	.37	.33	---	.48
M7	.62	.70	---	1.65

* Experiments B1 and B2 did not contain an initial control period.

"---" basal growth hormone concentration not present.

TABLE XII: Total Glucocorticoids in Plasma: B1-B6 (ug/dl)

SAMPLE #	TIME	*B1	*B2	B3	B4	B5	B6
1 A&B	0			1.07	1.46	1.39	.55
22	60			1.15	.242	1.16	1.42
45	150			1.11	1.75	2.83	3.98
71	230			1.90	0.00	.59	.59
97	310			.85	.51	3.08	.20
127	410			0.00	.87	.65	2.63
1 A&B	0	1.33	.87				
18	60	2.58	2.43				
44	140	.47	0.74				
70	220	1.53	.16				
96	300	0.00	1.03				

* experiments B1 and B2 did not contain an initial control period, which is reflected in the different glucocorticoid sampling times. Although glucocorticoid levels fluctuated during the course of an experiment they could not be related to external manipulations.

TABLE XIII: Glucocorticoids in Plasma; B7, B8 and M series (ug/dl)

SAMPLE #	TIME	*B7	*B8	M1	M2	M3	M4	M5	M6	M7
1 A&B	0	2.01	1.40	1.81	0.72	1.13	0.17	1.09	2.16	.82
22	60	1.38	1.46	1.04	0	0	1.62	0	2.48	0
45	150	1.33	2.39	1.02	3.39	0.02	0.56	0.55	0.96	6.15
71	230	1.27	1.65	3.28	2.74	3.01	1.74	0.93	0.64	7.67
97	310	1.16	1.39	0.68	1.06	2.43	0.88	0.09	1.25	2.79
128	407.5	1.28	1.45	1.16	0.24	0.89	0.50	0.19	0.62	3.49
133	420	1.03	1.29	0.87	3.22	0.55	0.82	0.79	0.83	5.19
136	430	1.23	1.19	0.68	2.55	0.69	0.50	0.45	0	2.67
138	440	.96	1.02	1.11	1.25	0.60	0.50	3.07	0.92	2.21
146	460	1.30	1.73	3.16	0.14	0.99	0.70	2.85	0.21	.90
151	480	1.23	1.19	1.26	1.64	0.94	1.45	1.97	1.12	.29
159	500	1.46	1.45	1.69	2.21.	1.80	1.08	3.14	1.32	1.23
164	520	1.56	1.28	1.16	1.25	0.99	1.48	2.78	1.02	0.49
166	530	1.56	1.17	1.21	1.25	1.85	1.19	2.93	0.83	---

* experiments B7 and B8 are included here because they followed the same experimental protocol as the M series, but with a somatostatin Ab (soma #8) later shown to be less effective in vivo than some #10.

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