A possible role for the brain renin-angiotensin system in the regulation of LH secretion

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STEELE, MARIANNE K., ROBERT V. GALLO, AND WILLIAM F. GANONG. *A possible role for the brain renin-angiotensin system in the regulation of LH secretion.* Am. J. Physiol. 245 (Regulatory Integrative Comp. Physiol. 14): R805–R810, 1983.—To investigate the possibility that angiotensin II (ANG II) is involved in the regulation of luteinizing hormone (LH) secretion, ANG II was injected intraventricularly and intravenously into proestrous rats. Intraventricular ANG II caused an increase in plasma LH concentrations in 10 min, whereas intravenous ANG II in the same dose had no significant effect. Intraventricular administration of the ANG II antagonist, saralasin, during the afternoon of proestrus induced a significant decrease in the number of rats ovulating and abolished the ovulatory surge in LH secretion seen in saline-injected control animals. Intravenous saralasin in two different doses failed to inhibit ovulation and produced only a small decrease in the LH surge. In animals treated with intraventricular saralasin, the increase in plasma LH concentration produced by intravenous injection of 50 ng of LH-releasing hormone (LHRH) was normal, indicating no alteration in the sensitivity of the gonadotrops to LHRH. Intraventricular administration of the converting-enzyme inhibitor, enalapril diacid, inhibited ovulation and the ovulatory surge, whereas systemically administered enalapril diacid had no effect on LH secretion. The data suggest that ANG II generated in the brain may play a significant role in the regulation of LH secretion on the day of proestrus.

angiotensin II; ovulation; rat

THE OCTAPEPTIDE ANGIOTENSIN II (ANG II) has long been known to participate in blood pressure control and regulation of fluid intake (31). It also increases the secretion of ACTH and vasopressin (22, 23). Recent data suggest that ANG II may also affect the release of prolactin, growth hormone, and luteinizing hormone (LH) (29, 30). The effect of ANG II on LH secretion appeared to be dependent on the presence of estrogen, because central, and to a lesser extent peripheral, injections of ANG II increased plasma LH titers in ovariectomized, estrogen-primed rats, whereas ANG II had no effect on LH secretion in untreated ovariectomized animals. Evidence for effects of estrogen on other functions of the peripheral and central renin-angiotensin systems have been reported by others (8, 14, 16, 26).

There is considerable evidence that ANG II is produced in the brain (10, 22) and possibly in the anterior pituitary (17, 25, 28), as well as in the circulatory system. The present experiments were designed to test the hypothesis that endogenous brain and/or pituitary ANG II is involved in the regulation of LH secretion. The LH response to intraventricular or intravenous ANG II was tested in intact female rats on the day of proestrus, a time when the central nervous system and the pituitary gland have been endogenously "primed" by increasing circulating levels of estradiol starting on diestrus and peaking early on proestrus. In addition, the role of endogenous ANG II in the preovulatory LH surge was assessed by blockade of either central or peripheral ANG II receptors with saralasin during the afternoon of proestrus. To demonstrate that saralasin did not interfere with the response of the gonadotrops to LH-releasing hormone (LHRH), the LH response to exogenous LHRH was tested after intraventricular saralasin. Finally, the involvement of ANG II in the LH surge was investigated by blocking synthesis of ANG II on proestrus through peripheral or intraventricular administration of the converting-enzyme inhibitor, enalapril diacid.

METHODS

Animals. Mature female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing 250–300 g were housed singly with controlled lighting (lights on 0500–1900) and ad libitum access to Purina rat chow and tap water. Vaginal smears were taken daily. Only those animals exhibiting three consecutive 4-day cycles were used.

Intraventricular injections. Under methohexital sodium (Brevital, Lilly) anesthesia (5 mg/100 g), rats were implanted with chronic third ventricular cannulas made of 23-gauge stainless steel tubing, 17 mm in length. A stylette was inserted into each cannula to prevent obstruction, and the location of the cannula was confirmed when cerebrospinal fluid flowed from the cannula after the stylette was removed. After surgery, the cannulas were checked daily and the stylette cleaned, if necessary, with distilled water. One week after surgery, daily vaginal smears commenced. Injections of test substances or vehicle were made using a 25-μl Hamilton syringe connected by polyethylene tubing (PE-50) to a 30-gauge stainless steel injection needle that fit within and extended to the lower tip of the permanent third ventricular cannula. Before each injection, the tubing and needle were filled with the solution to be injected. The stylette of the cannula was removed just before and replaced immediately after the injection; total injection time was

about 3 min. The animals were not restrained during the injection and were generally undisturbed by the procedure. The regular handling of the animals and cleaning of the cannula apparently had habituated the animals to the injection protocol.

Jugular vein cannulation. Before 0900 on proestrus, a Silastic cannula was implanted in the jugular vein under ether anesthesia. The distal end of the cannula was passed subcutaneously and led through the skin between the scapulae. The rats were then returned to their individual cages. An extension of polyethylene tubing (PE-50, 30 cm in length) filled with heparin (500 U/ml) in 0.9% NaCl was attached to each cannula and passed to the outside of the cage. This cannula was used for peripheral administration of test materials and for withdrawal of blood samples for hormone determination.

Blood samples. Blood samples were collected without additional anticoagulant from the jugular vein while the animals were conscious and freely moving in their cages. The volume of all samples (0.4 ml) was replaced immediately after each bleeding by an equivalent volume of saline followed by 100 μl of heparinized saline. Plasma was separated by centrifugation at 4°C and stored frozen until the day of assay.

Radioimmunoassay. Plasma concentrations of LH were measured in duplicate using a protocol adapted from Niswender et al. (18), and the results were expressed as nanograms per milliliter in terms of the RP-1 reference standard. Some hormone levels were determined using the RP-2 standard, and the results were recalculated and expressed according to the RP-1 values (RP-2 $\times$ 61 = RP-1).

Ova counts. Ovulation was determined by removing the ovaries and oviducts from each animal between 0930 and 1100 on estrus. The oviducts were separated from the ovaries, and the ova were freed from the mass of cumulus cells and counted under a dissecting microscope. When seven or more ova were present, ovulation was judged to have been complete.

Statistics. Hormone values across time between groups were analyzed using analysis of variance for repeated measures followed by the Student-Newman-Keuls test. Frequency of ovulation between groups was tested using a chi-square analysis.

Protocols. The effects of intraventricular or intravenous injection of ANG II (50 ng) or saline were tested between 1000 and 1200 on the day of proestrus. A baseline blood sample was withdrawn 10 min before injection, and subsequent blood samples were taken 10, 20, and 50 min after injection. Water intake was monitored in this experiment. A preweighed and filled water bottle was placed on each cage immediately after drug or saline administration and left in place until the last blood sample was drawn. The difference between beginning and ending bottle weight (in grams) was recorded as water intake.

To test the effect of ANG II receptor blockade, bolus injections of saralasin (10 μg in 3 μl of saline intraventricularly, or 10 μg in 100 μl saline every hour or 20 μg in 100 μl saline every 30 min intravenously) were started at 1200 and continued until 1700 on the afternoon of proestrus. This intraventricular dose of saralasin has been reported to inhibit drinking for up to 90 min (31), with no significant effects on blood pressure in normal rats (20). In proestrous female rats, multiple intraventricular injections of saralasin according to the protocol described above did not modify mean arterial blood pressure and blocked the pressor response to intraventricular injection of 50 ng ANG II for at least 45 min (M. Steele, unpublished data). Control proestrous female rats were injected with saline intraventricularly (3 μl, $n = 5$) or intravenously (100 μl, $n = 5$). Blood samples to determine the presence or absence of the LH surge were taken at hourly intervals between 1230 and 1730. On the following day, a vaginal smear was taken to confirm the day of estrous cycle and the ova, if any, were counted.

Two additional groups of animals were used to test the possibility that centrally administered saralasin might reach the pituitary gland and alter the responsiveness of pituitary LHRH receptors. One group of animals received saralasin (10 μg in 3 μl of saline intraventricularly), and the other received saline (3 μl). Fifteen minutes later, LHRH (50 ng/100 μl of saline) was injected into the jugular vein. Blood samples were withdrawn immediately before the intraventricular injection, just before the injection of LHRH, and 10, 20, and 50 min after LHRH. The dose of LHRH used produces a submaximal increase in LH secretion (C. Blake, personal communication), and any change in responsiveness would presumably have been seen. All sampling was performed between 1000 and 1200 on proestrus.

To test the effect of inhibiting converting enzyme, enalapril diacid (MK421 diacid, Merck, Sharp and Dohme; 100 μg/4 μl intraventricularly or 1 mg/kg intravenously) was injected at 0930, 1300, and 1630 on proestrus. Control rats received saline intraventricularly (4 μl, $n = 3$) or intravenously (100 μl, $n = 5$). The central dose of 100 μg of enalapril diacid and the timing of the injections were selected after preliminary experiments in which it was found that the drinking response to intraventricular ANG I (75 ng) was completely abolished 2 h after intraventricular injection of 100 μg of enalapril diacid. Five hours after enalapril diacid, the inhibition was incomplete. In contrast, intraventricular injection of 50 μg of the diacid or 50 or 100 μg of the prodrug, enalapril, 2 h before ANG I administration did not significantly affect water intake or frequency of drinking (M. Steele, unpublished observations). Similar data on the effectiveness and duration of converting-enzyme blockade by central enalapril or enalapril diacid, using the pressor response to ANG I, have recently been published (32). The peripheral dose of enalapril diacid used in the present experiment is sufficient to block converting-enzyme activity in plasma for 4–6 h (12), whereas equivalent doses given orally do not affect converting-enzyme activity in whole brain (7). Blood samples were taken at hourly intervals starting from 1230 to 1730. On the following day (estrus), a vaginal smear was taken and the ova, if any, counted.

RESULTS

In the rats tested on the morning of proestrus, plasma LH concentrations were significantly greater 10 and 20

min after intraventricular injection of ANG II than after injection of saline (Table 1). After intravenous administration of ANG II, hormone values tended to increase, particularly at the 20-min time point (Table 1). However, this value was not statistically different from either base-line levels or saline time-control values, which also showed a small increase. Water intake was increased by intraventricular injection of ANG II but not by saline (ANG II: 3.4 ± 0.7 g in 50 min vs. saline: 0.7 ± 0.3 g; $P < 0.05$). Intravenous administration of this dose of ANG II did not produce a significant increase in fluid ingestion (ANG II: 0.2 ± 0.2 vs. saline: 0.4 ± 0.3 g).

TABLE 1. *Plasma LH concentration before and after intraventricular or intravenous ANG II or saline vehicle on the morning of proestrus*

	Time Before or After Injection, min			
	−10	+10	+20	+50
	Intraventicular			
ANG II*	25.3 ± 4.2	43.5 ± 7.3†	38.8 ± 7.7†	29.6 ± 1.8
Saline	22.0 ± 4.1	21.2 ± 1.3	23.0 ± 4.2	25.7 ± 3.1
	Intravenous			
ANG II*	39.9 ± 8.5	44.0 ± 8.1	61.3 ± 8.0	37.9 ± 4.6
Saline	33.1 ± 4.8	38.7 ± 4.7	43.1 ± 10.3	31.6 ± 6.2

Values are means ± SE, expressed in ng/ml; $n = 6$ rats/group. LH, luteinizing hormone; ANG II, angiotensin II. * 50 ng. † $P < 0.05$ vs. base-line and time-control values.

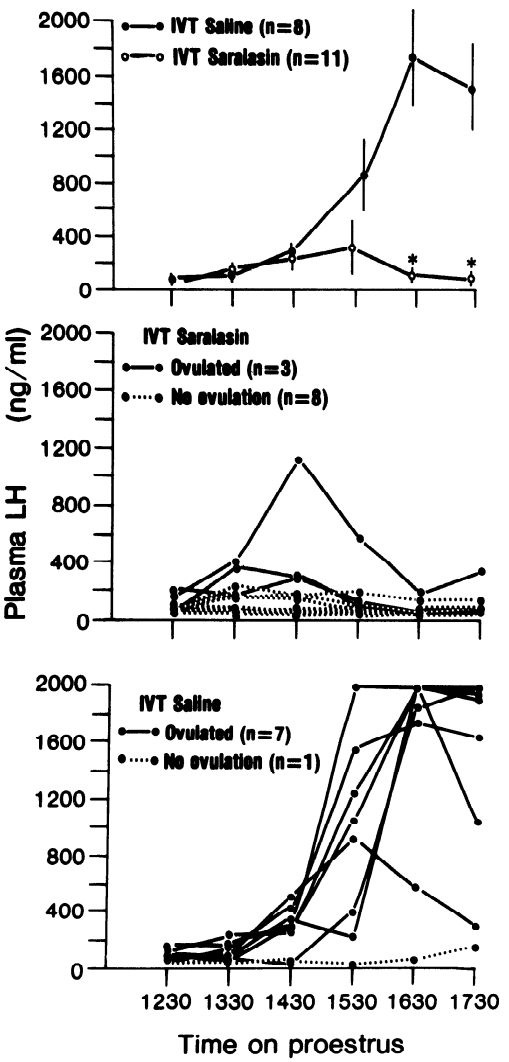

FIG. 1. Effect of intraventricular (IVT) saralasin (10 μg/3 μl, *open circles*) or saline (*closed circles*) on plasma luteinizing hormone (LH) concn in proestrous rats. Hourly injections of saralasin were started at 1200. *Top*: mean values; *middle*: individual values correlated with presence (*solid lines*) or absence (*dashed lines*) of ovulation in rats given saralasin; *bottom*: individual values in rats given saline. *$P <$ 0.01 vs. saline.

The timing and the magnitude of the LH surge were not different in rats treated with saline intraventricularly, whether the protocol was that of saralasin or of enalapril diacid, and were similar to that described previously (4). Consequently the results in the two groups that received saline intraventricularly were pooled (Figs. 1 and 3). Similarly the results in the two groups that received saline intravenously were comparable, and they were also combined (Tables 2 and 3).

Central injections of saralasin during the afternoon of proestrus blocked the LH surge, when mean levels of plasma LH were compared between experimental and control animals (Fig. 1, top). Analysis of the hormone profiles of individual saline-injected animals revealed that plasma LH concentrations were increasing by 1430 and had attained their highest levels by 1630 (Fig. 1, bottom). Plasma LH values from saralasin-injected animals remained low (Fig. 1, middle). Ovulation occurred in 7 of 8 saline-injected animals compared with 3 of 11 animals given saralasin ($\chi^2 = 7.8$, $P < 0.05$). In this and all subsequent experiments, ovulation was either complete (i.e., ≥7 ova) or did not occur at all.

Intravenous administration of saralasin (10 μg/h or 20 μg/0.5 h) did not significantly affect the mean levels of plasma LH attained during most of the surge, compared

TABLE 2. *Plasma LH concentration at various times after intravenous saline or saralasin on proestrus*

	n	Time					
		1230	1330	1430	1530	1630	1730
Saralasin							
10 μg/h × 6	6	65 ±6	63 ±10	76 ±15	883 ±400	1,254 ±435	880* ±204
20 μg/0.5 h × 12	6	58 ±7	58 ±57	273 ±138	798 ±431	914 ±252	722* ±224
Saline	10	80 ±13	115 ±25	224 ±44	520 ±118	1,455 ±264	1,543 ±254

Values are means ± SE, expressed in ng/ml; n, no. of rats/group. LH, luteinizing hormone. Hourly or half-hourly injections of saralasin were started at 1200. * $P < 0.05$ vs. time-control saline value.

TABLE 3. *Plasma LH concentration at various times after intravenous saline or enalapril diacid on proestrus*

	n	Time					
		1230	1330	1430	1530	1630	1730
Saline	10	80 ±23	115 ±25	224 ±44	520 ±118	1,455 ±264	1,543 ±354
Enalapril diacid, 1.0 mg/kg	11	45 ±10	82 ±21	141 ±41	525 ±240	895 ±235	1,053 ±439

Values are means ± SE, expressed in ng/ml; n, no. of rats/group. LH, luteinizing hormone. Enalapril diacid was administered at 0930, 1300, and 1630.

with values from saline-injected animals (Table 2), although the plasma LH concentrations in the last samples were significantly lower than the concentrations in saline-injected animals. The incidence of ovulation was not modified by intravenous saralasin at either dose level (saline: 9 of 10 ovulated; saralasin, 10 μg/h: 5 of 6 ovulated; saralasin, 20 μg/0.05 h: 4 of 6 ovulated; χ^2 = 1.8, not significant).

The possibility that centrally injected saralasin would modify the responsiveness of pituitary LHRH receptors was not supported; plasma LH levels after intravenous administration of LHRH in rats receiving intraventricular saralasin were the same as in the animals pretreated with saline (Fig. 2).

The LH surge was abolished by intraventricular enalapril diacid (Fig. 3, top) when groups means were compared with control values. Inspection of individual animal's profiles, however, revealed that four animals treated with intraventricular converting-enzyme inhibitor did show a proestrous rise in LH (Fig. 3, middle). Two of these surges were advanced relative to the timing seen in saline-injected animals (Fig. 3, bottom), whereas two animals that ovulated apparently had a delayed LH surge. The incidence of ovulation in rats treated with intraventricular enalapril diacid was significantly different from that seen in saline-injected animals (enalapril: 6 of 11; saline: 7 of 8; χ^2 = 3.9, $P < 0.05$). Peripheral administration of enalapril diacid did not significantly affect either the LH surge (Table 3) or the frequency of ovulation, compared with values of saline-treated controls (saline: 9 of 10 ovulated; χ^2 = 1.3, nonsignificant).

DISCUSSION

In the present experiments, intraventricular ANG II stimulated LH secretion on the morning of proestrus, whereas intraventricular administration of the angiotensin receptor-blocking drug, saralasin, or the converting-enzyme inhibitor, enalapril diacid, inhibited the proestrous LH surge and blocked ovulation. In addition, centrally administered saralasin did not interfere with the response of the gonadotrops to LHRH. Taken together, these data suggest that ANG II produced in the brain

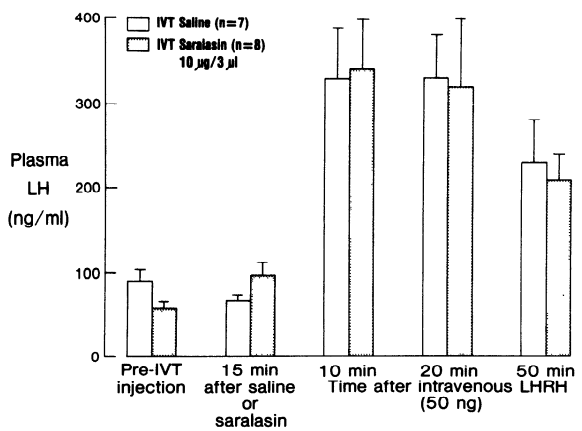

FIG. 2. Lack of effect of intraventricular (IVT) saralasin on plasma luteinizing hormone (LH) response to intravenous LH-releasing hormone (LHRH).

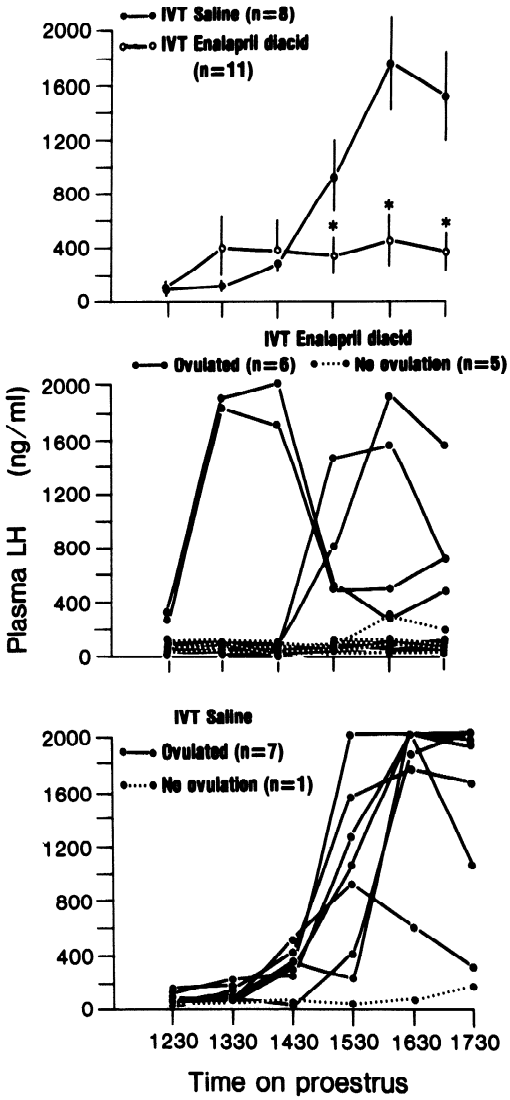

FIG. 3. Effect of intraventricular (IVT) enalapril diacid (100 μg/4 μl, *open circles*) or saline (*closed circles*) on plasma luteinizing hormone (LH) concn in proestrous rats. Injections of enalapril diacid were given at 0930, 1300, and 1630. *Top*: mean values; *middle*: individual values correlated with presence (*solid line*) or absence (*dashed line*) of ovulation in rats given saralasin; *bottom*: individual values in rats given saline. *$P < 0.01$ vs. saline.

exerts a significant effect on the secretion of LH from the pituitary gland of female rats on the day of proestrus.

Angiotensinlike immunoreactivity has been localized in gonadotrops (29), and the enzymes necessary for the local manufacture of ANG II have been demonstrated in the pituitary gland (17, 25). Despite some debate, it now seems clear that there is a brain renin-angiotensin system (10, 22), and neurons containing ANG II-like immunoreactivity end in the external layer of the median eminence (11). Therefore it is possible that ANG II secreted from the brain into the portal vessels or ANG II produced within the pituitary gland itself might affect adenohypophysial LH secretion at the level of the pituitary gland. However, the pituitary gland is outside the blood-brain barrier, and neither intravenous ANG II nor intravenous enalapril diacid produced significant changes in LH secretion. Further, intravenous saralasin

had only a slight, delayed effect. It was previously reported (30) that intravenous ANG II stimulated LH secretion, but the increase was small and the dose administered was 1,000 times the dose used in the present experiments. In addition, a small increase in LH release was reported after 3 h of incubation of pituitary quarters with a large dose of ANG II (28). However, three laboratories have now reported that ANG II has no effect on LH release from anterior pituitary cells in vitro (1, 13, 27). Consequently a direct stimulatory effect of ANG II on LH secretion from the anterior pituitary gland seems unlikely.

Another possibility is that centrally generated ANG II could effect LH secretion by acting on the circumventricular organs. Lesions of the organum vasculosum of the lamina terminalis (21, 34) or of the subfornical organ (15) have been reported to affect estrous cyclicity, as well as the incidence and magnitude of the LH surge. However, intravenously administered ANG II should penetrate the circumventricular organs and produce an increase in LH secretion, and intravenous saralasin should block the receptors in the circumventricular organs that would trigger LH secretion. In our experiments intravenous ANG II did not affect LH release, and intravenous saralasin had only a slight, delayed effect. Consequently an effect via the circumventricular organs seems unlikely.

ANG II could affect the release of LHRH at brain sites other than the circumventricular organs, e.g., the preoptic area. This effect could be either directly on the LHRH neurons or through an intervening mechanism. One inviting hypothesis with some experimental basis is that ANG II affects LH secretion by alterations in the secretion of catecholaminergic neurons. ANG II acts on noradrenergic neurons in the autonomic nervous system to facilitate norepinephrine release (24, 35), and evidence is accumulating that it also facilitates the release of norepinephrine and dopamine in the brain, including the preoptic area (2, 6, 9). Centrally released norepinephrine stimulates LH secretion (3, 33), and the preoptic area is innervated by noradrenergic neurons (19) and contains LHRH-secreting neurons (3). Furthermore ANG II receptors in the preoptic area increase in number during proestrus (5). Therefore it is possible that ANG II facilitates the release of LHRH by causing release of norepinephrine in the preoptic area. Of course, ANG II could be acting directly on LHRH neurons in the hypothalamus, thereby eliciting LH release.

Taken together, the data from the present series of experiments demonstrate that in the presence of estrogen, ANG II can increase the release of LH from the anterior pituitary gland. The attenuation of the LH surge after central ANG II receptor blockade or after inhibition of central nervous system converting-enzyme activity support the hypothesis that an endogenous renin-ANG II system exists in the brain and that this system is involved in the production of the LH surge on the afternoon of proestrus in female rats.

The assistance of Anna Varberg Reisator is greatly appreciated.

This work was supported by the Kroc Foundation and National Institutes of Health Grants AM-06704, AM-07265, HD-05577, and HD-05492. The enalapril and enalapril diacid used in these studies were generously provided by Merck, Sharp and Dohme, West Point, NJ.

Received 7 March 1983; accepted in final form 20 June 1983.

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