

# Brain regulation of gastric secretion: Influence of neuropeptides

(bombesin/ $\beta$ -endorphin/central nervous system/gastric acid output)

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**ABSTRACT** Several neuropeptides injected intracisternally were assessed for their effects on gastric secretion in rats. Bombesin (1  $\mu$ g) completely suppressed gastric acid secretion, reduced the volume of gastric secretion, and partially blocked insulin- or 2-deoxy-D-glucose-induced stimulation of gastric acid output. The inhibitory effect of this peptide is dose-dependent, long-acting, reversible, and specific. Bombesin response appears to be central nervous system-mediated; its expression is not dependent on the vagus nerve or the adrenal glands, and does not rely on a decrease in gastrin secretion. Among seven other peptides tested, only  $\beta$ -endorphin and a potent gonadotropin releasing-factor (gonadoliberein) agonist significantly reduced gastric acid secretion, with an activity *ca.* 100 times less than that of bombesin. The presence of bombesin-like material in rat brain and the high potency of bombesin to inhibit gastric secretion suggest that this peptide may be of physiologic significance as a chemical messenger involved in brain modulation of gastric secretion.

The original observation that discrete brain lesions induced pathological changes in the gastric mucosa (1, 2) focused attention on the role of the central nervous system in the pathophysiological regulation of gastric function. Subsequently, numerous investigators attempted to elucidate brain structures and neurogenic/humoral pathways involved in the control of gastric secretion. In rats, several studies have demonstrated that the hypothalamus influences gastric secretion. Stimulation of the lateral hypothalamus, but not of the surrounding area, caused a vagally mediated increase in acid output (3, 4). On the other hand, stimulation of the ventromedial hypothalamus decreased acid secretion (3), whereas lesion experiments had opposite effects (5). Previous studies of chemical signals participating in the brain modulation of gastric secretion seem to indicate that cholinergic-stimulatory (6) and noradrenergic-inhibitory mechanisms play a role (4, 7).

The presence in mammalian brain of oligopeptides acting in the central nervous system to influence glucoregulation (8, 9), thermoregulation (10, 11), blood pressure (12, 13), sympathetic outflow (9), and stress-induced gastric hemorrhagic lesions (14) led us to studies designed to assess the roles these peptides may play in brain control of gastric secretion. The results indicate that bombesin\*, a tetradecapeptide originally isolated from anurian skin (15), injected intracerebrally is extraordinarily potent in inhibiting gastric acid secretion.

## MATERIALS AND METHODS

Male Sprague-Dawley CD rats (250-300 g), maintained ad lib on Purina Laboratory Chow and tap water, were housed under conditions of controlled temperature and illumination (from 06:00 to 22:00 hr). All experiments were performed according to the same time schedule in 24-hr-food-deprived rats with free access to water up to the beginning of treatment.

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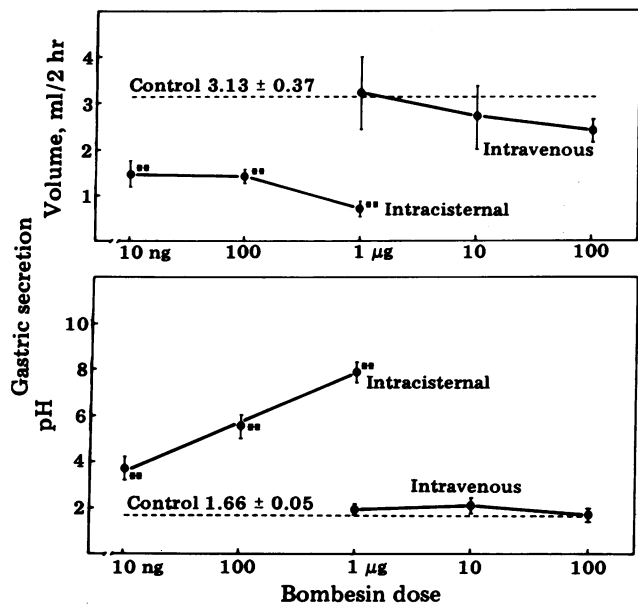


FIG. 1. Linear dose-response curve for i.c. injection of bombesin. Fasted rats (24 hr) were injected i.c. or i.v. with saline or various doses of bombesin and the pylorus was ligated. Two hours later, rats were sacrificed for collection of gastrin secretion. Each point represents the mean  $\pm$  SEM of 5-17 animals. The degree of significance of the difference between bombesin and saline (control) given i.c. is shown as \*\* =  $P < 0.01$ .

Rats under light ether anesthesia were injected intracisternally (i.c.) or intracerebroventricularly (i.v.t.) through a permanent cannula implanted into the right lateral brain ventricle a few days before, or intravenously (i.v.) through the jugular vein, with saline or test materials. Immediately after injection, the pylorus portion of the stomach was ligated. The righting reflex was normally regained 5-10 min after surgery, and conscious rats were decapitated 2 hr following pylorus ligation unless otherwise specified. Trunk blood and gastric content were collected separately and centrifuged. Plasma glucose concentrations were determined by the glucose oxidase method with a Beckman glucose analyzer. Gastric secretory volume and pH were measured and samples (0.5 or 1 ml) were analyzed for gastric acidity by titration with 0.01 M sodium hydroxide to a pH of 7.0. A bilateral adrenalectomy or a sham operation was performed immediately before peptide injection and pylorus ligation. Subdiaphragmatic vagotomy by using the general method described by Martin *et al.* (16) was performed 1 week prior to the experiments.

In some studies, intra-atrial silastic catheters were placed and a permanent cannula was implanted into the right lateral brain

Abbreviations: GnRF, gonadotropin releasing-factor (gonadoliberein); i.c., intracisternal(ly); i.v.t., intracerebroventricular(ly); i.v., intravenous(ly); 2-dDG, 2-deoxy-D-glucose.

\* Bombesin has the following structure: Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH<sub>2</sub>.

Table 1. Gastric secretory response to i.c. injection of oligopeptides

Treatment	Rats	Gastric secretion			
		Volume, ml/rat	pH	Titratable acidity	
				Concentration, meq/liter	Output, $\mu\text{eq}/2\text{ hr}$
Saline	12	2.3 $\pm$ 0.4	1.7 $\pm$ 0.1	71 $\pm$ 5	160 $\pm$ 34
Bombesin	8	0.7 $\pm$ 0.2**	7.9 $\pm$ 0.4**	0**	0**
[D-Trp <sup>8</sup> ]Bombesin	8	2.2 $\pm$ 0.3	1.5 $\pm$ 0.1	68 $\pm$ 5	154 $\pm$ 27
$\beta$ -Endorphin	10	2.1 $\pm$ 0.3	2.4 $\pm$ 0.3*	47 $\pm$ 7*	107 $\pm$ 24
GnRF	8	2.2 $\pm$ 0.4	2.0 $\pm$ 0.2	57 $\pm$ 8	123 $\pm$ 27
[D-Trp <sup>6</sup> ,Pro <sup>9</sup> -NEt]GnRF	10	0.9 $\pm$ 0.1*	2.0 $\pm$ 0.2	56 $\pm$ 6	58 $\pm$ 8*
Somatostatin	5	2.5 $\pm$ 0.5	2.1 $\pm$ 0.3	66 $\pm$ 4	173 $\pm$ 40
Neurotensin	9	2.5 $\pm$ 0.3	2.2 $\pm$ 0.3	73 $\pm$ 9	203 $\pm$ 42
Substance P	7	1.9 $\pm$ 0.3	1.7 $\pm$ 0.1	64 $\pm$ 6	175 $\pm$ 42

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$  as compared with saline-treated rats. Fasted rats (24 hr) under light ether anesthesia were injected intracisternally with saline (10  $\mu\text{l}$ ) or peptides (1  $\mu\text{g}/10\ \mu\text{l}$ ) and the pylorus was ligated. Two hours later, the animals were decapitated and the gastric secretion was collected.

ventricle as described (17). After 5–7 days, rats were injected i.v.t. with saline or bombesin. Blood samples were withdrawn before the injection and at 15-min intervals for 1 hr (17). The plasma was separated and frozen for subsequent gastrin determinations, according to the method of Walsh (18). A guinea pig antiserum to porcine gastrin at the final dilution of 1:100,000 (provided by John Walsh, UCLA) was used, and synthetic human gastrin-17 was utilized for iodination and as an unlabeled reference standard.

Peptides were synthesized using solid phase methodology (19). Insulin (Eli Lilly) and 2-deoxy-D-glucose (2-dDG) (Schwarz BioResearch, Orangeburg, NY) were freshly dissolved in 0.9% saline before use and injected in 10- $\mu\text{l}$  (i.c.) or 0.2-ml (i.v.) volumes.

Following analysis of variance, differences between groups were determined by the multiple range test of Dunnett and Duncan by using the computer program EXBIOL.

## RESULTS

Table 1 shows that bombesin administered i.c. (1- $\mu\text{g}$  dose) markedly reduced the volume of gastric secretion, raised pH values to 7.9, and completely inhibited acid secretion as measured 2 hr after peptide injection and pylorus ligation. Among the other peptides tested under the same conditions (Table 1) or at higher doses (5–10  $\mu\text{g}$  i.c., data not shown), [D-Trp<sup>8</sup>]bombesin, neurotensin, somatostatin, gonadotropin releasing-factor (GnRF), and substance P did not affect gastric secretion, whereas  $\beta$ -endorphin reduced titratable acid concentration and [D-Trp<sup>6</sup>,Pro<sup>9</sup>-NEt]GnRF decreased the volume and acid output

of gastric secretion. A higher dose of  $\beta$ -endorphin (5  $\mu\text{g}$  i.c.) did not increase the inhibitory response (data not shown).

The comparison of i.c. and i.v. dose-response curves to bombesin in 2-hr-pylorus-ligated rats (Fig. 1) indicated that the peptide, when given i.c., was very potent in decreasing the volume and titratable acidity of gastric secretion in a dose-dependent manner and in increasing pH values with a linear dose-response relationship within the dose range of 10–1000 ng. The minimal effective dose was less than 10 ng (6 pmol). Inhibition of gastric secretion also was observed when bombesin was administered via the lateral ventricle of rat brain (data not shown). By contrast, systemic injection of the peptide, even at doses  $10^4$  times higher than that which was effective when administered i.c., had no effect.

The time course of gastric response to a 100-ng dose of bombesin given i.c. is shown in Fig. 2. pH values are significantly elevated and the volume of secretion is markedly reduced during the first 2 hr following peptide injection; later these parameters become similar to that of saline-treated controls.

The effect of bombesin (1  $\mu\text{g}$  i.c.) on gastric acid output stimulated by 2-dDG and insulin is depicted in Fig. 3. In 2-hr-pylorus-ligated rats, bombesin inhibited gastric acid response to 2-dDG by 70% and to insulin by 55%.

Bombesin injected i.v.t. with a 1- $\mu\text{g}$  dose (but not with a 100-ng dose) significantly increased plasma levels of gastrin, as measured for 60 min following peptide injection to conscious, chronically (i.v.t. and intra-atrial) cannulated rats (Fig. 4).

In sham-operated rats, bombesin (1  $\mu\text{g}$  i.c.) increased plasma

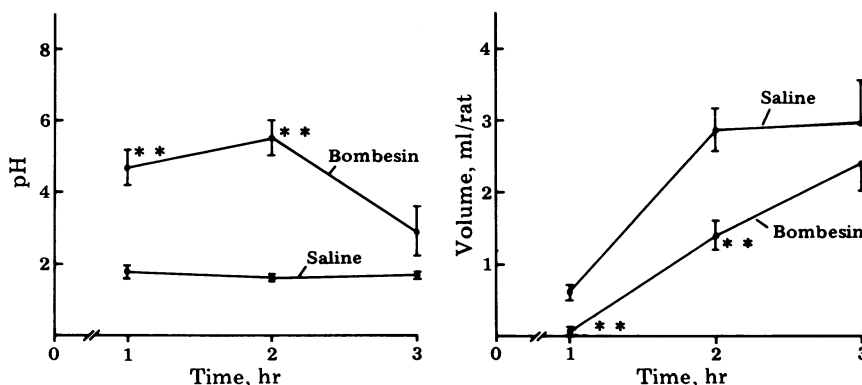


FIG. 2. Temporal effect of bombesin on gastric secretion. Fasted rats (24 hr) were injected i.c. with saline or bombesin (100 ng) and the pylorus was ligated. One, 2, or 3 hr later, the animals were decapitated. For other details, see the legend of Fig. 1. \*\* =  $P < 0.01$ .

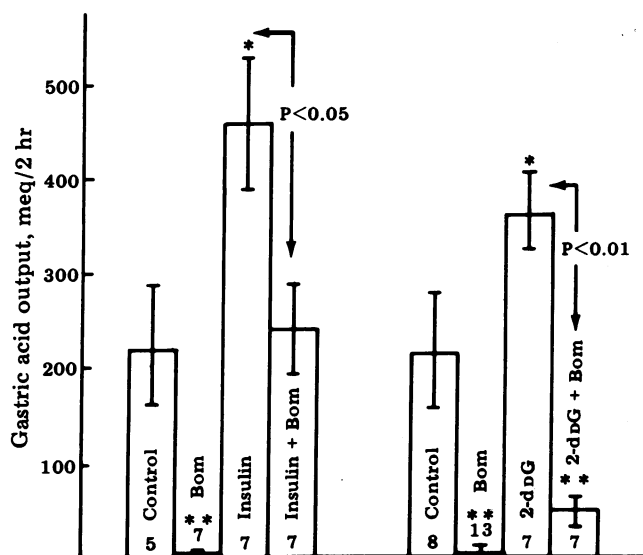


FIG. 3. Inhibitory effect of bombesin on gastric acid output stimulated by insulin and 2-dDG. Fasted rats (24 hr) were injected with saline (i.v. or i.c.), insulin (1 unit, i.v.), or 2-dDG (3 mg, i.c.) and then with saline (i.c.) or bombesin (Bom, 1  $\mu$ g, i.c.), after which the pylorus was ligated. Two hours later, the animals were decapitated. The columns represent the mean  $\pm$  SEM. The number of animals in each group is shown at the base of each column.

glucose levels, reduced the volume of gastric secretion, and suppressed gastric acidity (Table 2). Adrenalectomy completely prevented the hyperglycemic effect of the peptide but did not abolish its inhibitory action on gastric secretion. Vagotomy did not alter the bombesin-induced rise in pH of gastric secretion (Fig. 5).

## DISCUSSION

The present results show that some oligopeptides or their analogs have the capability of influencing gastric secretion when injected into the brain. Of particular interest was the demonstration that i.c. injection of bombesin (1  $\mu$ g) in pylorus-ligated rats results in a complete suppression of gastric acid output and in a marked reduction of the secretory volume. The bombesin effect is dose-dependent, with a minimal effective dose less than 10 ng, and is long-acting and reversible, as shown by the normalization of gastric secretion 3 hr after peptide injection (100 ng).

The specificity of bombesin's action is demonstrated by the inactivity of analog [D-Trp<sup>8</sup>]bombesin and of other unrelated peptides, such as neurotensin, substance P, GnRF and somatostatin. The absence of a neurotensin effect on gastric secretion is at variance with the report of Osumi *et al.* (20) that showed a decrease in gastric acid output after i.v.t. injection of neuro-

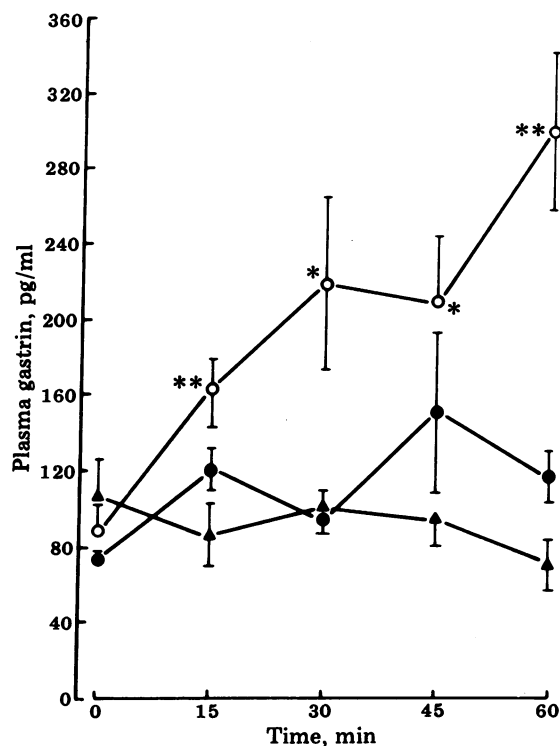


FIG. 4. Temporal effects of bombesin (1  $\mu$ g or 100 ng) given i.v.t. on plasma gastrin levels in chronically intra-atrial and intraventricularly cannulated 24-hr-fasted rats. Each point represents the mean  $\pm$  SEM of 4–8 animals. O, 1  $\mu$ g of bombesin (eight rats); ●, 100 ng of bombesin (four rats); ▲, saline (seven rats). \* =  $P < 0.05$ ; \*\* =  $P < 0.01$  as compared with the saline-treated group.

tensin in rats. Variations in the route of peptide injection, anesthesia procedure, and methods of collection of gastric secretion may be involved in the difference observed.  $\beta$ -Endorphin has been shown to share a number of common central nervous system-mediated actions with bombesin (14, 21, 22), and it is not unexpected that the opioid peptide also reduced gastric acid secretion. Brodie *et al.* (23) have observed that i.v.t. injection of morphine (8–32  $\mu$ g) decreased gastric volume and titratable acidity in 2-hr-pylorus-ligated rats. The fact that GnRF was inactive in influencing gastric secretion whereas the agonist produced a significant response could be related to the potency of the analog (24–26). Bombesin appears *ca.* 100–250 times more potent in suppressing gastric secretion than any other peptide tested (Table 1).

Our experiments indicate also that bombesin inhibits gastric secretion by initially acting in the central nervous system, because the peptide did not influence gastric secretion after i.v. administration at doses  $10^4$  times higher than those that were

Table 2. Inhibitory effect of bombesin given i.c. on gastric secretion in adrenalectomized rats

Treatment	Rats	Glucose, mg/dl	Volume, ml/rat	pH	Gastric secretion	
					Concentration, meq/liter	Output, $\mu$ eq/2 hr
1. Sham with saline	10	114 $\pm$ 6	1.9 $\pm$ 0.3	2.0 $\pm$ 0.2	65 $\pm$ 7	136 $\pm$ 34
2. Sham with bombesin	9	195 $\pm$ 14**	0.7 $\pm$ 0.1**	7.8 $\pm$ 0.4**	0 $\pm$ 0**	0 $\pm$ 0**
3. Adr-x with saline	9	79 $\pm$ 3	2.8 $\pm$ 0.6	2.0 $\pm$ 0.3	77 $\pm$ 10	265 $\pm$ 65
4. Adr-x with bombesin	14	94 $\pm$ 5	1.9 $\pm$ 0.3*	4.2 $\pm$ 0.6**	29 $\pm$ 6**	67 $\pm$ 17**
		(**)	(*)	(**)	(**)	(*)

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$  as compared with their respective controls (groups 2 vs. 1 and 4 vs. 3). Symbols in parentheses are in comparison with group 2. Fasted rats (24 hr) under ether anesthesia were sham-operated or bilaterally adrenalectomized, injected i.c. with saline or bombesin (1  $\mu$ g/10  $\mu$ l), and the pylorus was ligated. Two hours after pylorus ligation, the animals were decapitated. Adr-x, bilateral adrenalectomy.

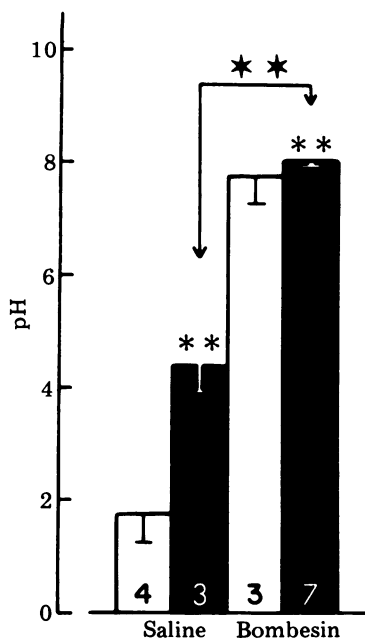


FIG. 5. Effect of bombesin on the pH of gastric secretion in vagotomized rats. Sham ( $\square$ ) or vagotomized ( $\blacksquare$ ) 24-hr-fasted rats were injected i.c. with saline or bombesin ( $1 \mu\text{g}$ ) and the pylorus was ligated. Two hours later the rats were decapitated. For other details, see the legend of Fig. 3.

effective i.c. In agreement with the latter finding, bombesin administered i.v. or subcutaneously has been reported to have no influence on acid secretion in the perfused preparation of rat stomach (27), whereas bombesin is a very potent gastric acid releaser in dog or in human (28, 29). The exact neuroanatomical site of bombesin action, perhaps located in structures known to affect gastric secretions and food intake or those already demonstrated to be the substrate mediating its hypothermic action (30), is presently unknown.

Gastrin and the parasympathetic nervous system are well-established stimulatory factors in the control of gastric secretion (6), and we evaluated if bombesin action involved the inhibition of such humoral/neurogenic pathways. The results show that bombesin action is not mediated through a decrease in gastrin release, because intracerebral injection of the peptide at a  $100\text{-ng}$  or  $1\text{-}\mu\text{g}$  dose resulted respectively in no change or in an elevation in plasma gastrin levels. The release of gastrin could be related to the rise of gastric pH leading to the removal of gastric acid-mediated inhibition of gastrin secretion. Vagal tone has a critical role in the production of gastric secretion in pylorus-ligated rats (31), and secretagogues, such as 2-dDG and insulin, act through a cephalic vagal stimulation (32, 33). The fact that bombesin suppressed basal and insulin- or 2-dDG-stimulated gastric secretion in pylorus-ligated rats suggests that the peptide could decrease vagal activity. However, whereas in pylorus-ligated rats vagotomy is well-known to decrease gastric acid secretions (23, 31, present observation), bombesin was shown to completely suppress acid secretion. Such a difference in the magnitude of the inhibitory effect and the fact that vagotomy did not alter gastric response to bombesin suggest that the vagus nerve did not play an important role in the mechanisms that mediated bombesin action.

The demonstrated central nervous system-mediated effects of bombesin included the development of a sustained hyperglycemia (8) and hyperglucagonemia (21), which is adrenal-dependent through an increase in sympathetic outflow (8). Because it has been shown that hyperglycemia (34, 35), hyperglucagonemia (36, 37), and increased sympathetic outflow

(4, 7) singly decrease gastric acid secretion in experimental animals and in humans, we evaluated the influence of bombesin on gastric secretion in the absence of such alterations. It is evident that in adrenalectomized rats, bombesin still produced a significant inhibition in both the acidity and volume of gastric secretion, whereas the hyperglycemia response was abolished (33, present observation). These results support the concept that the effect of bombesin on gastric secretion is not adrenal-dependent although the stimulation of adrenomedullary secretion induced by bombesin (8) could contribute in part to the peripheral expression of its antisecretory action. More research is needed to determine the cerebral and peripheral neurogenic/humoral processes through which bombesin injected into the brain induced such alterations of gastric secretion.

These studies raise the question as to whether a bombesin-like substance plays a role in mammalian brain control of gastric secretion. Bombesin is now recognized to have immunologically characterized counterparts in mammalian brain and gastrointestinal tract (38). Recently, McDonald *et al.* (39) have isolated from porcine gut a 27-amino acid peptide with the C-terminal decapeptide fragment essentially identical to the C-terminal decapeptide of frog skin bombesin. Such a peptide has been shown to mimic several central nervous system-mediated actions of bombesin on glucoregulation, thermoregulation (40), and gastric secretion (41).

Moreover, high-affinity binding sites for bombesin have been characterized in rat brain synaptosomal preparations (42), and the release of bombesin-like peptide from hypothalamic slices induced by depolarizing stimuli have been reported (43). The presence of bombesin-like activity in brain tissue and its clear effectiveness at very low doses suggest that this peptide may have physiologic significance as a central nervous system chemical messenger participating in cerebral modulation of gastric secretion.

In conclusion, the demonstration that some oligopeptides are able to act in the central nervous system to influence gastric secretion makes them suitable tools to dissect the cerebral and peripheral control of such function. It also raises the possibility that some of them may participate in the biochemical processes involved in central nervous system regulation of gastric secretion under physiologic and pathologic circumstances.

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