

*Current Concepts:*

IV. EFFECTS OF BOMBESIN ON BEHAVIOR

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Summary

This report describes the influence of bombesin on the gross behavior of goldfish, frogs, mice, rats, guinea pigs, rabbits, chicks, pigeons and monkeys. Goldfish, frogs, chicks and pigeons were overtly unaffected by bombesin given centrally and/or peripherally. Mice, rats, guinea pigs, rabbits and monkeys responded quickly to intracerebroventricular (i.c.v.) and/or intrathecal (i.th.) administration of bombesin by displaying a range of behaviors suggestive of altered skin sensation. In mice, bombesin was essentially equipotent as a scratch inducer by i.c.v. and i.th. routes ( $A_{50} = 0.010-0.019 \mu\text{g}$ ) but 6800 times less potent i.p. In rats, bombesin-induced grooming and scratching behaviors were shown to be qualitatively different from those associated with ACTH-(1-24) and thyrotropin releasing hormone. Spantide and [D-Arg<sup>1</sup>, D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>, Leu<sup>11</sup>] substance P (both at 0.20, 0.50 and 0.80  $\mu\text{g}$  i.c.v.), two proposed bombesin receptor antagonists, did not markedly influence bombesin-induced scratching or hypothermia in rats.

Bombesin is a tetradecapeptide which was originally isolated from frog skin (1). In keeping with its designation as a brain-gut peptide, bombesin promotes homeostasis (2) by regulating gastrointestinal function (3-5), food intake (6-8), blood glucose levels (9,10) and body temperature (11-13).

Mice and rats have been mainly used in studies involving bombesin and behavior. For example, central injection of bombesin to rats increases locomotor activity when the animals are tested in small Plexiglas chambers (14). The increased locomotion can be antagonized if the animals are pretreated with neuroleptics such as haloperidol or fluphenazine (15). The nature of the testing environment is important since bombesin suppresses ambulation (and rearing) if the rats are run in an open-field apparatus (16).

The most obvious response of mice (17-20) and rats (11,21-23) to i.c.v. bombesin is a remarkable display of grooming and scratching during which the animals seem preoccupied with skin sensation. Does bombesin affect the ongoing behavior of other species in a similarly dramatic fashion? This question provided the stimulus for the present investigation. Additionally, quantitative inform-

ation is provided on scratching in mice when bombesin is given by one of three routes - i.c.v., i.th. and i.p. Second, qualitative information is provided on scratching in rats by comparing the behavioral syndromes associated with bombesin, adrenocorticotropin [specifically, ACTH-(1-24)] (24) and thyrotropin releasing hormone (TRH)(25). And third, two proposed bombesin receptor antagonists, spantide ([D-Arg<sup>1</sup>, D-Trp<sup>7,9</sup>, Leu<sup>11</sup>]substance P) (26) and DAPTL-SP ([D-Arg<sup>1</sup>, D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>, Leu<sup>11</sup>]substance P (27) are tested against bombesin-induced scratching and hypothermia in rats.

#### Materials and Methods

Compounds Naloxone HCl (kindly supplied by Endo) was dissolved in saline. The following peptides were dissolved in distilled water immediately before injection: ACTH-(1-24) (courtesy of H. Kuijs, Organon, Holland); bombesin (Sigma); DAPTL-SP (Bachem); spantide (Peninsula) and TRH (Sigma).

Goldfish Eight goldfish of the comet variety, weighing 3-5 g, were obtained from a local supplier. They were housed in a tank (under constant aeration) in a laboratory for 5 days. A standard light-dark cycle was maintained with a timer-regulated light period from 07.00 hr to 19.00 hr. Experiments took place between 10.00 hr and noon. Bombesin (5  $\mu$ l) was injected s.c. in the belly. The doses were 0.10  $\mu$ g (n=2), 5  $\mu$ g (n=2) and 25  $\mu$ g (8.3 mg/kg; n=4). The goldfish were observed for 2 hr post-injection.

Frogs Male frogs (*Rana pipiens*; 25-40 g) were purchased from West Jersey Biological Supply Farm, Wenonah, N.J. Two days before experimentation, they were weighed and placed in individual plastic observation boxes (25 cm long; 20 cm wide; 15 cm high) containing water to a depth of 1 cm. Environmental temperature was maintained at 20 $\pm$ 0.5 $^{\circ}$ C by means of an automatic thermostat. Lights were on between 9.00 hr and 18.00 hr. Bombesin was given i.th. at the articulation between the seventh and eighth vertebrae (28) (0.10  $\mu$ g in a volume of 1  $\mu$ l; n=6) or s.c. (0.25 ml/25 g; 10 mg/kg; n=4). The frogs were observed over the following 1 hr.

Mice Male ICR mice (22-28 g) (Temple University Skin and Cancer Hospital) were each placed in an individual Plexiglas observation box (22 cm long; 18 cm wide; 25 cm high) and allowed to habituate for at least 15 min. They were injected with bombesin i.th. (29) (5  $\mu$ l), i.c.v. (30) (5  $\mu$ l) or i.p. (0.25 ml/25 g) and immediately observed (i.c.v. and i.th.), or observed at +5 min (i.p.), for 15 min. Four mice were scored simultaneously. The grooming and scratching shown by each animal was monitored for 5 sec out of every 20 sec. A positive score was given if the mouse groomed or scratched during each interval. Results are presented as percent of the maximum (i.e., 45) possible number of grooming/scratching episodes (%MGE). Four mice were used per dose of bombesin. A<sub>50</sub> values were estimated by linear regression analysis (31). Experiments took place between 14.00 hr and 18.00 hr.

Rats Male Sprague Dawley albino rats (180-200 g; Zivic-Miller) were anesthetized with ketamine HCl (100 mg/kg i.p.) and placed in a Kopf stereotaxic frame. A polyethylene cannula (PE 10) was positioned in a lateral cerebral ventricle of each rat as previously described (32). After surgery, the animals were housed individually and allowed 5-7 days for recovery prior to i.c.v.

administration of ACTH-(1-24), bombesin or TRH (each agent in 5  $\mu$ l water then a 3  $\mu$ l wash with more water). When appropriate, DAPTLS-SP, spantide or water was given i.c.v. 5 min before a standard dose of bombesin (0.10  $\mu$ g i.c.v.) or water. Behavior of rats (n=10) was monitored (beginning 5 min after each bombesin injection) for 5 sec out of every 20 sec over a 30 min observation period and quantitated as previously described (33). Cannula placements were verified after each experiment by injecting methylene blue and checking for distribution within the cerebroventricular system.

Additional rats were infused with bombesin (0.18  $\mu$ g/hr for 7 days) (23) to the caudal region of the Sylvian aqueduct from a s.c. implanted osmotic minipump (Alzet 2001) (34), then challenged with DAPTLS-SP (0.5  $\mu$ g i.c.v., n=3) and observed for 30 min.

The possibility that spantide and/or DAPTLS-SP might prevent bombesin-induced hypothermia was studied. Rats (n=5) were each placed in an individual Plexiglas observation box (26 cm long; 20 cm wide; 30 cm high) and allowed to habituate to a temperature of  $6\pm 2^\circ\text{C}$  for 1 hr (11). Rectal temperatures were taken several times with a thermistor probe to familiarize the animals with the procedure. The baseline (zero time) temperature was noted (with the probe inserted 6 cm into the rectum) then test agent or water was given i.c.v. 20 min before bombesin (0.10  $\mu$ g i.c.v.) or water. Temperatures were taken at 30, 60, 90 and 120 min.

In a tolerance experiment, additional rats (n=6) received a continuous i.c.v. infusion of water (1  $\mu$ l/hr) or bombesin (0.18  $\mu$ g/hr) (23) from a s.c. implanted Alzet 2001 minipump. After 7 days, they were challenged with bombesin (0.10  $\mu$ g i.c.v.) and rectal temperatures were taken at an ambient temperature of  $6\pm 2^\circ\text{C}$  as outlined above.

Guinea pigs Male albino guinea pigs (n=4) (180-200 g; Charles River) were each cannulated in the lateral cerebral ventricle (35) in a manner similar to that described for rats. They lived in individual Plexiglas boxes (26 cm long; 20 cm wide; 30 cm high) and were observed in these boxes during the 60 min after injection of bombesin (0.50  $\mu$ g in 5  $\mu$ l water).

Rabbits Three male New Zealand albino rabbits (2-4 kg; Biosearch, Philadelphia) were anesthetized with pentobarbital (30 mg/kg i.p.). A polyethylene cannula was inserted into the left lateral cerebroventricle (36) and behavioral experiments started 5 days later. Bombesin (0.10  $\mu$ g) was given i.c.v., in 100  $\mu$ l of water. This was followed by a 100  $\mu$ l wash with more water. Rabbits were placed in trash cans (diameter: 35 cm; height: 55 cm) with which they had been familiarized and their gross behavior was observed for 1 hr.

Chicks Groups of 5 white leghorn cockerels (2-3 days old; Moyers Chicks, Quakertown, PA) were placed in open makrolon observation boxes. The floor of each box was covered with sawdust. Water and food crumbs were made available throughout the experiment. After a 30 min control period, each chick was injected s.c. on one side of the abdomen with water (0.25 ml/25 g) or bombesin (1, 5 and 12.5 mg/kg). The overt behavior of the birds was observed for 60 min. The study was repeated with different chicks (n=5) when they were 11 days old.

Pigeons Two White Carneau pigeons were selected from the group of birds being used in the evaluation of psychoactive agents in the Department of Pharmacology, University of Michigan. Both pigeons had previously received a variety of compounds. One dose of bombesin (100  $\mu$ g) was given i.c.v. (37) in the present study and the birds were observed for the following 60 min.

Monkeys Four rhesus monkeys (4-6 kg) were chosen from the colony of morphine-dependent animals being used to assess the physical dependence liability of new analgesics at the University of Michigan. A stainless steel cannula was implanted in a lateral cerebral ventricle of each monkey as previously described (38,39). (Cannula placements were subsequently verified by radiography). Two doses of bombesin (750  $\mu$ g and 1 mg) were tested in the monkeys (n=2) about 2 hr after their regular maintenance dose of morphine (3 mg/kg s.c.). The animals were observed for the ensuing 4 hr.

### Results

Bombesin caused no overt effects when given to goldfish (0.10, 5 and 25  $\mu$ g s.c.), frogs (0.10  $\mu$ g i.th. and 10 mg/kg s.c.), chicks (1, 5 and 12.5 mg/kg s.c.) and pigeons (100  $\mu$ g i.c.v.).

The most obvious behavior induced by centrally administered bombesin in mice was an immediate and incessant scratching of the flanks and neck area with the hindpaws (Fig. 1A). Facial grooming with the forepaws was also greatly increased. Oral preening of the tail (Fig. 2A) was frequent when bombesin was given i.th.; less so when given i.c.v.; and infrequent when given i.p. Scratching/grooming was dose-related (Fig. 3).  $A_{50}$  values (and 95% confidence limits) for bombesin by i.c.v., i.th. and i.p. routes were 0.010  $\mu$ g (0.003-0.030) [i.e. 6 pmole (1.8-18)], 0.019  $\mu$ g (0.010-0.30) and 68  $\mu$ g (32-147), respectively.

In rats, bombesin (0.05, 0.10 and 0.50  $\mu$ g) caused excessive scratching of the head and neck area by the hindpaws within 1-3 min of injection (Fig. 1B). The rats frequently licked their hindpaws before scratching. Facial grooming (with forepaws), body washing (with tongue and teeth), forepaw tremor, stretching and wet-dog shaking also occurred. These behaviors lasted 45-60 min.

TRH and ACTH-(1-24) also caused increased grooming, forepaw tremor and body shaking in rats but these syndromes lacked the compulsive scratching component so characteristic of bombesin. TRH (3 and 10  $\mu$ g i.c.v.) provoked extensive face washing (Fig. 1C), licking the front paws and oral stereotypies. These behaviors appeared within 5 min of injection and lasted 10-20 min. The increased grooming associated with ACTH-(1-24) (1.5 and 3  $\mu$ g i.c.v.) in rats was similar to normal grooming in that the rats thoroughly washed the face and entire body (Fig. 1D) but the bouts were longer. Onset time was 12-17 min and the effects lasted about 1 hr.

DAPT-SP and spantide (both at 0.20, 0.50 and 0.80  $\mu$ g i.c.v.) were tested against the grooming/scratching caused by bombesin in rats. Higher doses of DAPT-SP and spantide (e.g. 2  $\mu$ g) elicited behavioral effects which precluded further testing. These effects were immediate prostration and barrel rotation. Even at 0.50 and 0.80  $\mu$ g, both agents provoked, on occasion, barrel rotation and motor impairment. These animals were rejected. Neither DAPT-SP nor spantide attenuated bombesin-induced grooming/scratching.

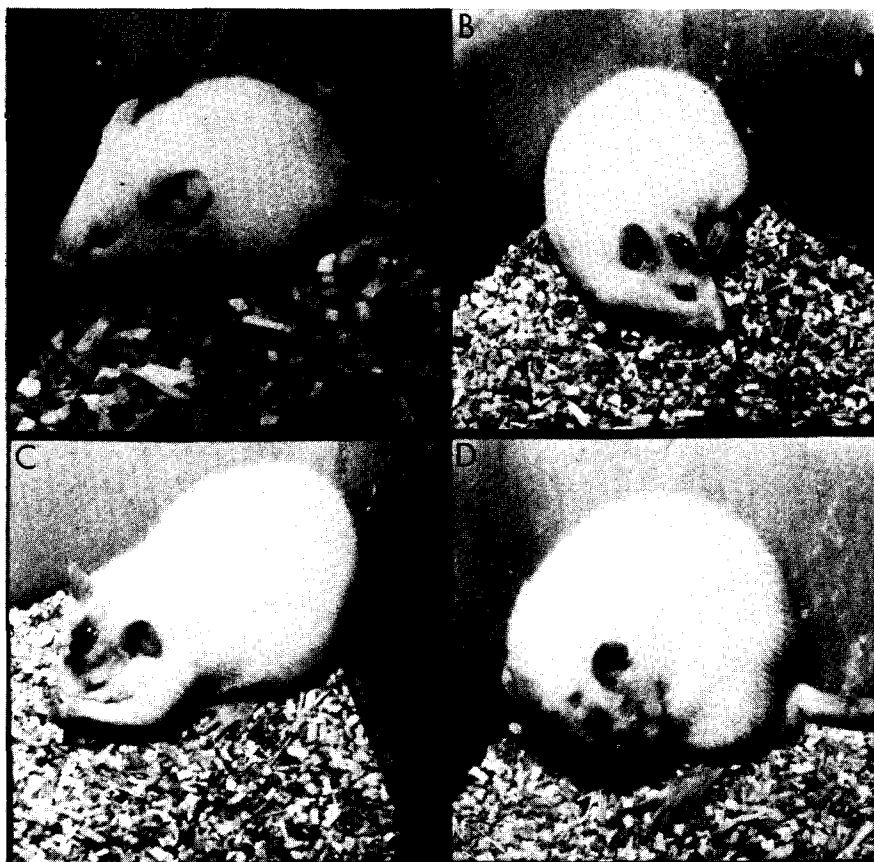


FIG. 1

Scratching and grooming behavior in mouse (A) and rat (B-D). (A) Bombesin, 0.02  $\mu\text{g}$  i.th. (B) Bombesin, 0.10  $\mu\text{g}$  i.c.v. (C) TRH, 10  $\mu\text{g}$  i.c.v. (D) ACTH, 3  $\mu\text{g}$  i.c.v.



FIG. 2

Grooming and scratching behavior in mouse (A) and guinea pig (B). (A) Bombesin, 0.02  $\mu\text{g}$  i.th. (b) Bombesin, 0.50  $\mu\text{g}$  i.c.v.

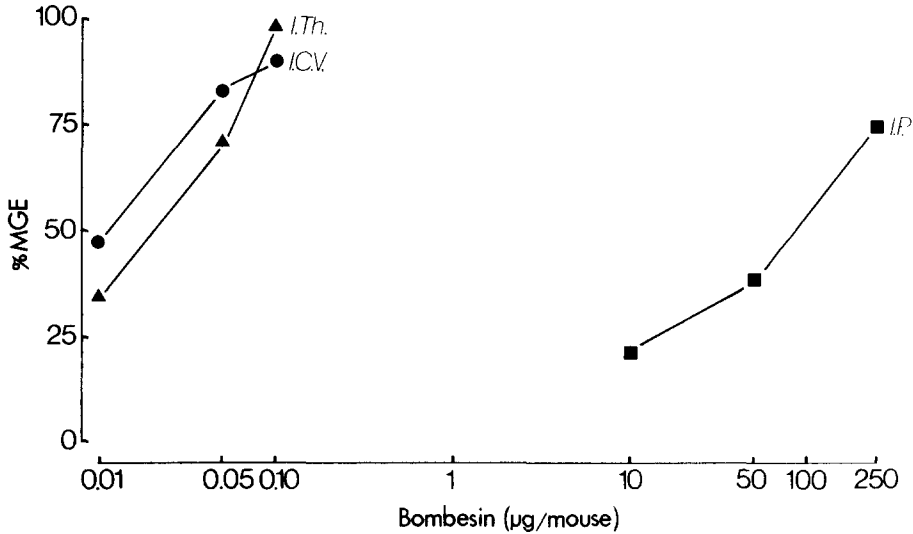


FIG. 3

Grooming/scratching induced in mice ( $n=4$ ) by intracerebroventricular, intrathecal and intraperitoneal administration of bombesin. %MGE is the percent of the maximum possible number of grooming/scratching episodes in 15 min.

DAPTIL-SP (0.50 µg i.c.v.) evoked no overt change in behavior when given to rats that had received a central infusion of bombesin for 7 days.

DAPTIL-SP and spantide (both at 0.20, 0.50 and 0.80 µg i.c.v.) were again ineffective as bombesin antagonists against a second endpoint - bombesin-induced hypothermia in rats placed in an environment of  $6\pm 2^\circ\text{C}$ .

It is known that tolerance does not develop to the scratching associated with bombesin administration to rats (23,40). This was confirmed in the present study and contrasts with the demonstrated development of tolerance to bombesin-induced hypothermia (in the same rats) at  $6\pm 2^\circ\text{C}$  (Fig. 4).

In guinea pigs, bombesin (0.50 µg i.c.v.) caused immediate behavioral activation. Within 3 min, all four animals displayed excessive scratching of the head and neck area with the hindpaws (Fig. 2B), face washing with forepaws and wet-dog shaking. One guinea pig stretched and writhed several times at +32 min. The syndrome lasted 30-45 min; thereafter, the animals were quiet and all showed ptosis.

Bombesin (0.10 µg i.c.v.) caused immediate behavioral agitation and rearing in all three rabbits studied. Within 2 min, the animals displayed face washing with forepaws. Between 10 and 30 min, face washing, scratching of the flanks and neck with the hindpaws, and wet-dog shaking, occurred every 3-4 min, that is, in a regular fashion but not so frequently as we observed in mice, rats and guinea pigs.

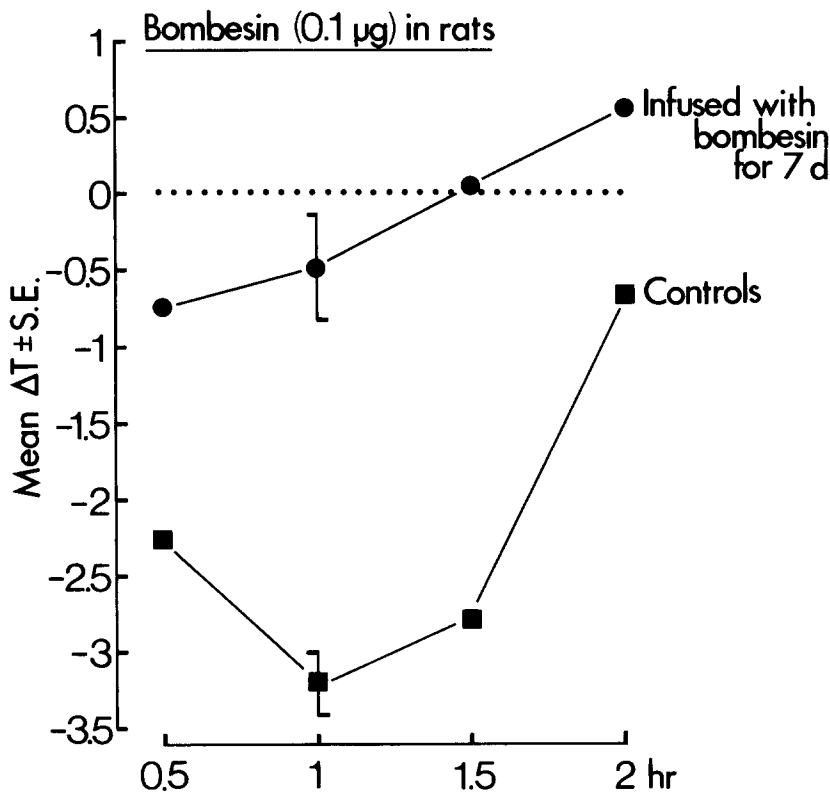


FIG. 4

Development of tolerance to the hypothermic effect of bombesin. Rats ( $n=6$ ) received an i.c.v. infusion of water (■) or bombesin (●) (0.18  $\mu$ g/hr) from a s.c. osmotic minipump. After 7 days, both groups received bombesin (0.10  $\mu$ g i.c.v.) and rectal temperatures were taken for 2 hr at  $6 \pm 2^\circ\text{C}$ .

Bombesin (750  $\mu$ g and 1 mg i.c.v.) altered the behavior of four rhesus monkeys in a dramatic manner. During injection of the peptide (with the monkey in a restraining chair), a transient redness appeared about the face (and particularly the eyes) of the animals. In the cage, they immediately spat out food that was stored in their cheeks, became agitated and restless and began pacing. Within 2-5 min, the monkeys rubbed their eyes with the back of their wrists. Thereafter, they scratched the face, ears, head, soles of feet, and entire body; and rubbed their face and palms along the bars of the cage. On average, one scratching/rubbing episode occurred per min (Fig. 5). There was evidence of abdominal discomfort (protecting the stomach, retching, vomiting) as well as piloerection, abnormal postures, tongue movements and hypothermia. The syndrome lasted at least 4 hr.

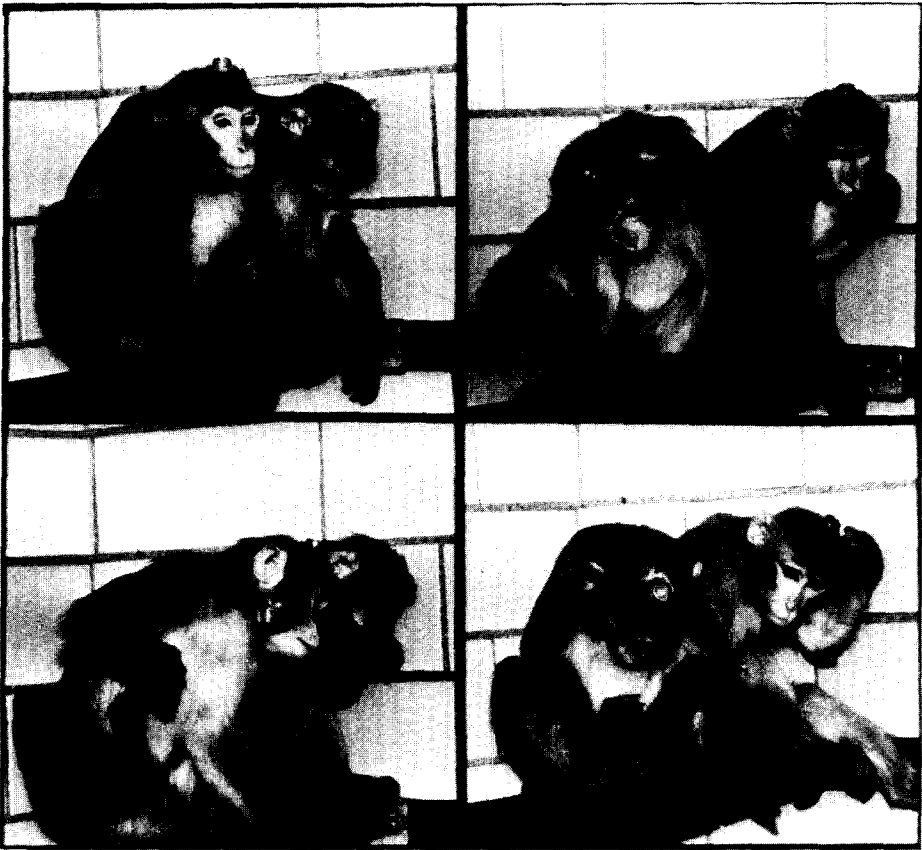


FIG. 5

Scratching/grooming behaviors in morphine-dependent rhesus monkeys 30-60 min after bombesin (750  $\mu\text{g}$  i.c.v.)

#### Discussion

Bombesin causes mice (17-20) and rats (11,21-23) to groom and scratch. The present experiments have shown that bombesin is a potent inducer of scratching/grooming in certain other animals as well. Thus, mice, rats, guinea pigs, rabbits and monkeys quickly respond to small doses of centrally administered bombesin with behaviors which, outwardly, are suggestive of changes in skin sensation. The overt behavior of goldfish, frogs, chicks and pigeons appears to be unaffected by the peptide, at least under our experimental conditions. In this context, it is of interest that locomotor activity of the teleost fish, Catostomus commersoni, is not markedly influenced by i.c.v. application of bombesin (5  $\mu\text{g}$ /g) (41). Also, De Caro et al. (42) studied bombesin in the pigeon at doses (0.01-1  $\mu\text{g}$  i.c.v.) lower than we used (100  $\mu\text{g}$  i.c.v.). These workers found bombesin to be dipsogenic, and behaviorally inactive, in this species.

In mice, bombesin is essentially equipotent as a scratch-inducer by i.c.v. ( $A_{50}$ =0.010  $\mu\text{g}$ ) and i.th. ( $A_{50}$ =0.019  $\mu\text{g}$ ) routes of administration. Scratching can be elicited by i.p. injection



but a dose 6800 times greater than the i.c.v. dose is required. O'Donohue et al. (20) studied bombesin after i.th. administration to mice and concluded that this peptide, like substance P, is a neurotransmitter of primary sensory afferents. Scratching induced by bombesin and substance P in mice can be differentiated pharmacologically since morphine attenuates the behavior associated with substance P (43) but not that caused by bombesin (17). It remains to be seen if agonists which show selectivity for kappa opioid receptors will antagonize bombesin-induced scratching in mice. This is certainly the case in rats where bombesin-induced scratching is antagonized in a stereospecific and dose-related manner by benzomorphan kappa agonists but not by many standard opioids and opioid peptides (44).

As in mice, bombesin acts at spinal, as well as supraspinal, levels in rats to cause scratching (45). The i.c.v. and i.th. A<sub>50</sub>'s for the peptide in mice and rats are comparable (45). The behavioral effects displayed by rats after bombesin can easily be distinguished from those provoked by ACTH-(1-24) and TRH (see results). What makes rats react in such a startling yet distinctive manner to these peptides? How and where does the animal perceive the stimulus that triggers the excessive behaviors? Why does tolerance not develop to bombesin-induced scratching just as it does to the hypothermia (46)? We do not have answers to these questions at the moment. The availability of bombesin antagonists that work in vivo would obviously help. The two agents tested in the present study - spantide (26) and DAPTL-SP (27) - proved disappointing. We were unable to demonstrate antagonism of bombesin-induced scratching or hypothermia over the dose range, 0.20-0.80 µg i.c.v. Higher doses caused barrel rotation (47) and motor impairment to such an extent that behavioral experiments were rendered meaningless. In contrast to our experience, Yachnis et al. (48) recently used a sample of spantide (2 µg i.c.v. - Peninsula) that gave no side effects and they were able to prevent bombesin-induced grooming and hypothermia in rats.

A 5-min infusion of bombesin (1-4 µg/kg) had no marked effect on the gross behavior of baboons (49). We found that bombesin caused extensive scratching in nonwithdrawn morphine-dependent rhesus monkeys after i.c.v. administration of 750 and 1000 µg. This behavior has previously been noted in monkeys undergoing withdrawal from certain kappa agonists (50). The other signs and symptoms observed in the present study - restlessness, abdominal discomfort, vomiting, piloerection, abnormal postures and hypothermia - may well be acute effects of bombesin in this species. They also constitute a passable morphine-like abstinence syndrome. Is bombesin precipitating true abstinence, quasi-abstinence (51) or a bit of both? Testing the peptide in drug-naive monkeys should provide the answers.

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