Table 1 Synthesis and secretion of C5 and total protein by mouse peritoneal macrophages

Strain	Serum	C5 antigen: Extracellular (c.p.m.)	C5 antigen: Intracellular (c.p.m.) High MW	C5 antigen: Intracellular (c.p.m.) Low MW	Total protein c.p.m. ($\times 10^{-3}$) Extracellular	Total protein c.p.m. ($\times 10^{-3}$) Intracellular
A/HeJ	–	−115	970	2,700	3.5	74.9
AKR	–	50	1,600	2,960	5.1	100
B10.D2/old	–	−5	2,080	4,220	8.2	101
DBA/2J	–	−170	3,430	5,730	12.8	129
SWR	–	80	3,600	7,900	8.9	168
Swiss	+	1,820	2,030	3,380	8.2	100

Sera from mice used for peritoneal macrophage cultures were tested for the presence of C5 antigen by Ouchterlony analysis (results in agreement with findings in ref. 3). Peritoneal cell cultures were established as described in Fig. 1 legend and the extracellular C5 antigen estimated in 125-μl aliquots by subtracting the radioactivity in control (ovalbumin/anti-ovalbumin) immunoprecipitates (mean 427 c.p.m. per 125 μl; range 523 for B10.D2/old line to 308 for AKR) from radioactivity in C5-anti-C5 immunoprecipitates. Intracellular C5 antigen content was estimated from the radioactivity in the high MW pro-C5 peak and low MW peak on SDS-polyacrylamide gel electrophoresis of C5 immunoprecipitates (representative pattern is shown in Fig. 2). To estimate total protein synthesis, TCA precipitation was performed on 5-μl aliquots as described in Fig. 1 legend.

chase of C5-deficient (A/HeJ) cells with medium containing unlabelled methionine resulted in little change in the intracellular C5 antigen pattern on SDS-polyacrylamide gel electrophoresis and no appearance of labelled C5 protein in the extracellular medium. Estimation of pool size and intracellular turnover of C5 antigen cannot be determined, but within the time limits of the experiments, even delayed secretion of labelled C5 antigen was ruled out.

The possibility was considered that macrophages from the deficient strains secreted an unstable C5 precursor protein or a proteolytic enzyme that destroyed normal pro-C5. Accordingly, intracellular C5 protein produced by and prepared from A/HeJ (C5-deficient) cells was incubated for 30 min at 37 °C with unlabelled, C5-deficient (A/HeJ) conditioned medium, C5-sufficient (Swiss) conditioned medium or fresh medium alone. No degradation of labelled pro-C5 (MW ~ 200,000) or the small MW (38,000) proteins was observed on SDS-polyacrylamide gel electrophoresis of the incubation mixtures.

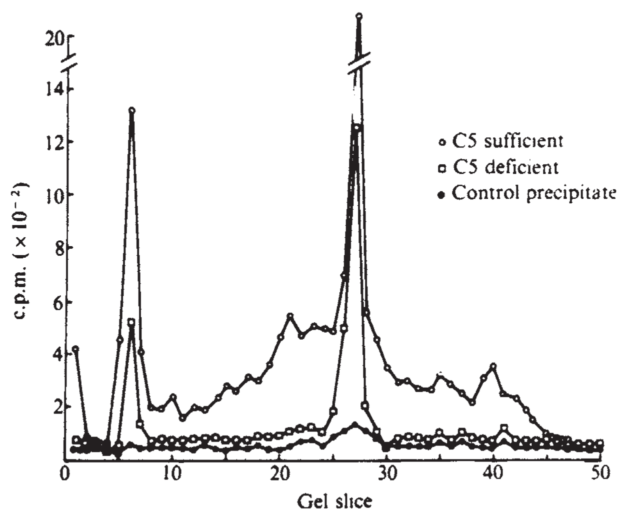

Fig. 2 SDS-polyacrylamide gel electrophoresis of radiolabelled reduced and alkylated C5 protein contained in cell lysates of mouse peritoneal cells from Swiss (C5-sufficient, ○), and A/HeJ (C5-deficient, □) strains. Radioactivity in control immunoprecipitates indicated by closed circles. Estimated MW of the large MW peak was 203,000. In other experiments, estimates ranged from 201,000 to 210,000. The low MW peak was 34,000–38,000. This low MW peak does not correspond in size to a known subunit of C5. A similar pattern of intracellular labelled C5 antigen (two peaks—205,000 and 38,000 MW) was observed in a detergent lysate of Swiss mouse spleen cells obtained from an animal injected with 5 mCi ^{35}S-methionine (supplied by Dr Allen Schwartz).

These data suggest that C5 deficiency in mice is due to a defect in secretion. This may result from a failure of glycosylation as has been proposed as a mechanism for a defect of secretion in certain human immunoglobulin deficiency diseases[8], a mutant mouse myeloma[9] and in normal plasma cells exposed to tunicamycin[10]. A missense mutation could lead to a lack of a suitable site for glycosylation on the C5 polypeptide chain. Alternatively, the defect might reside in synthesis of a protein required specifically for C5 glycosylation or for C5 secretion. In any case, these experiments indicate that the C5 mRNA is translated in cells from several mouse strains genetically deficient in C5, but that the product (pro-C5) is not secreted.

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Bombesin suppresses feeding in rats

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Bombesin (BBS) is a tetradecapeptide originally isolated from amphibian skin[1]. BBS-like immunoactivity is widely distributed in mammalian gut[2–5], and plasma levels have been shown to rise sharply following feeding (ref. 6 and V. Erspamer, personal communication). The physiological actions of BBS are unknown. We have previously shown that the classic gut hormone cholecystokinin (CCK) is a powerful and specific suppressor of food intake[7–9]. Although CCK and BBS lack common amino acid sequences, they have certain common actions on gut viscera[10,11]. We have now shown that BBS also suppresses food intake, and we compare its action with that of CCK.

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Eleven male Sprague–Dawley rats weighing 440–520 g were housed and tested in individual cages on an artificial 12-h light cycle. They were maintained on solid food pellets (Purina) overnight and tested with a balanced liquid food preparation (No. 116EC, GIBCO) diluted 1:3 with deionised water. Solid food was removed at 0930 h each morning, and rats were then allowed access to liquid food for 30 min. This pre-deprivation meal of liquid food assured that all rats had eaten a meal shortly before they were deprived of all food at 1000 h. Immediately before the presentation of liquid food in graduated drinking tubes for the test at 1300 h, rats were injected intraperitoneally (i.p.) with 1 ml of one of the following treatments on different days: (1) synthetic BBS in doses of 2, 4, 6, 8, 16 μg per kg body weight, diluted in 0.15 M NaCl; (2) synthetic C-terminal octapeptide of CCK (CCK8) in doses of 0.2, 0.4, 0.7, 1.4, 2.9 μg per kg, diluted in 0.15 M NaCl; (3) equivolumetric controls of 0.15 M NaCl. Food intake was measured at 5 and 15 min, and then at 15-min intervals for the remainder of the 180-min test. The behaviour of each rat was noted[12] during a 4-s interval every minute for the first 45 min of the test. This systematic observation of the behaviours of each animal permitted calculations of the incidences and pattern of the behaviours associated with feeding (exploration, grooming, apparent sleep), so that control and peptide-injected rats could be quantitatively compared; it also permitted the identification of any abnormal behaviour. In all tests, each animal served as its own control; statistical comparisons were made with a matched-pair t-test.

BBS and CCK8 each produced a large suppression of liquid food intake across the entire range of doses tested (Fig. 1). The smallest doses of each peptide produced equivalent 15% suppressions: 2 μg per kg BBS reduced food intake from a control of 18.8 ml to 15.9 ml ($P<0.01$); 0.2 μg (~4 Ivy units) per kg CCK8 reduced intake from 18.6 to 15.8 ml ($P<0.01$). The largest doses of each peptide also produced similar suppressions: 16 μg per kg BBS reduced intake from 20.5 ml to 8.1 ml ($P<0.001$); 2.9 μg (~63 Ivy units) per kg CCK8 reduced intake from 17.1 ml to 5.4 ml ($P<0.001$). A comparison of relative effectiveness on a molar basis revealed that the dose of BBS required to suppress feeding by 50% was five times greater than the equipotent dose of CCK8 (Fig. 1).

In a parallel experiment, the effects of BBS and CCK8 were also tested in animals given solid food (Purina rat pellets) following a 7 h (0900–1600 h) food deprivation. Both peptides reduced solid food intake. In these conditions, the lowest dose of BBS, 3 μg per kg, suppressed feeding by 21% (control intake, 2.8 g; intake after BBS, 2.2 g; $P<0.05$). The lowest dose of CCK8, 1.1 μg (~24 Ivy units) per kg suppressed feeding by 30% (from 2.7 g to 1.9 g; $P<0.01$). The largest dose of BBS tested, 6 μg per kg, suppressed feeding by 50% (from 2.8 g to 1.4 g; $P<0.01$). The largest dose of CCK8, 3.8 μg (~84 Ivy units) per kg, suppressed feeding by 77% (from 2.6 g to 0.6 g; $P<0.001$).

The actions of BBS and CCK8 were similar in two respects. Both peptides suppressed feeding rapidly, with maximum reductions occurring during the first 15 min after injection. In addition, both peptides produced transient suppressive effects: even after large doses, cumulative food intakes of peptide-injected rats had returned to normal levels within 120 min. However, the actions of the two peptides differed in one respect. Although both suppressed liquid and solid food intakes, CCK8 was two to five times as potent in suppressing liquid food intake as it was in suppressing solid food intake, a differential we have noted previously[7]. In contrast, BBS suppressed solid and liquid food intakes equally. Thus, the potency of CCK8 varied according to the physical characteristics of the test diet, whereas the potency of BBS did not.

As BBS suppressed solid as well as liquid food intakes, the suppression was not achieved through some interference with the motor acts required for licking. Furthermore, the largest dose of BBS tested had no effect on water consumption after the minimal water deprivation (6 h) required to achieve reliable intakes. Thus, BBS preferentially reduced feeding, not drinking, even when the same motor act (licking metal spouts for liquid food or water) was required for either ingestive behaviour; this differential strongly suggests that BBS did not reduce feeding simply by producing some generalised discomfort. Several further observations also indicated that BBS did not cause illness or distress. First, the rats never seemed acutely or chronically ill, and they gained weight normally during 8 months of frequent testing. Second, after injections of BBS, rats always began eating eagerly; they merely stopped sooner. Thus, no statistically significant differences in food intake between BBS- and saline-injected rats occurred during the first 5 min of feeding, but BBS-injected rats ate much less than saline-injected rats between 5 and 15 min of the test. This ineffectiveness of BBS in preventing or slowing the early phase of feeding was not an artefact of the time required for the distribution of exogenously injected BBS as it occurred whether the peptide was injected immediately before, 5 min before, or 15 min before food presentation. Thus, BBS preferentially acted to enhance satiety by shortening the meal; it did not prevent or delay the onset of a meal, nor did it slow the initial phase of eating. Third, analysis of the minute-by-minute behavioural data revealed that the only significant behavioural change produced by BBS was a decrease in the incidence of feeding (Table 1).

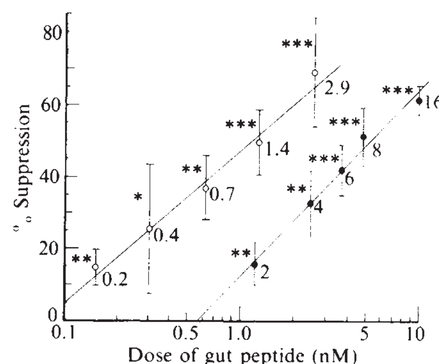

Fig. 1 Suppression of liquid food intake in rats (n = 11) during the first 15 min after i.p. injections of various doses of BBS (●) or CCK8 (○). The figure shows mean per cent suppressions and s.e.m. compared with intake on control tests. Doses of each peptide are in μg per kg. Doses of the two peptides on the abscissa are arranged along a molar logarithmic scale. Control intakes of liquid food ranged from 13.7 to 20.5 ml (mean 18.1 ml, s.e.m. 0.3 ml). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, statistical differences from respective 0.15 M NaCl controls. Correlation coefficients (Pearson product–moment): for BBS, $r=0.91$ ($P<0.025$); for CCK8, $r=0.97$ ($P<0.005$). Slopes of the regression lines were not significantly different[31] ($t=0.22$, $P>0.05$).

These data demonstrate that BBS did not reduce food intake simply by causing a generalised disruption of behaviour; rather, it produced a selective suppression of feeding, a quality which one would anticipate in a natural satiety signal. Furthermore, in no instance did BBS elicit any abnormal behaviour. Finally, in our unpublished observations, doses of BBS as high as 128 μg per kg have failed to produce any evidence of illness or distress in similar test conditions. Although intracisternal BBS can reduce core body temperature of cold-exposed rats[13], we found no change in core body temperature, as measured by rectal thermoprobe, 15 or 30 min after i.p. injections of doses of 4–32 μg per kg, the times when suppression of feeding was maximal.

The mediation of the satiety action of BBS is unknown. The slopes of the regression lines drawn for the BBS and CCK8 dose–response curves were not significantly different statistically (see Fig. 1). Parallelism of curves is consistent with but not proof of the same mechanism of action[14]. This parallelism might be explained if exogenous BBS produces its satiety effect simply by releasing endogenous CCK[10,15–17]. However, as described above, comparison of the dose–response curves for liquid and solid foods revealed a situation in which the effects of BBS and CCK8 diverged, depending on the physical characteristics of food ingested; this divergence demonstrates that the satiety

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Table 1 Incidence of behaviours occurring after i.p. injection of BBS (8 μg per kg) or vehicle control (0.15 M NaCl) in 11 rats

	Feeding	Exploration, grooming	Apparent sleep	Miscellaneous
Control	15	19	65	1
BBS	5*	23	71	1

Incidences are expressed as mean % of the total number of behavioural observations carried out. During the first 45 min of the test the only statistically significant effect which BBS produced was a reduction in the incidence of feeding.

* $P < 0.001$, BBS compared with control. All other BBS–control comparisons were nonsignificant ($P > 0.4$).

action of exogenous BBS cannot be mediated solely by endogenous CCK. Three observations of visceral gut actions of BBS relate to this point. (1) In humans, Basso *et al.*[11] have shown that the addition of secretin to a slow intravenous infusion of BBS caused a partial inhibition, rather than a potentiation, of BBS-induced pancreatic amylase output, suggesting that secondarily released CCK is not the major mediator of BBS-induced amylase output in these conditions. (2) Although enterectomy abolishes BBS-induced pancreatic protein secretion in the dog[15] it does not affect this action in the rat[18]. (3) BBS powerfully stimulates amylase release from *in vitro* preparations of rat pancreatic fragments[19,20] or dispersed acinar cells[21]. These findings strongly suggest that BBS can directly stimulate traditional CCK target tissues.

We conclude that BBS is a putative satiety signal. The existence of at least one unidentified gastric satiety signal has recently been demonstrated in the rat[22,23]. We have shown that the action of this gastric signal does not depend on vagal innervation[24], and may therefore be hormonal in nature. As it is present in large quantities in gastric mucosa[4], BBS is a candidate for this unidentified satiety signal.

We have not determined how the amount of BBS required to elicit satiety in this study relates to the amount and pattern of BBS released by a normal meal, nor how BBS may interact with other putative satiety signals, such as CCK. Finally, the site of action of BBS when it alters satiety behaviour is unknown, but it is of interest that BBS immunoactivity[4,5,25], like that of CCK[26–30], is present in large quantities in the brain.

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Human β-thromboglobulin inhibits PGI_2 production and binds to a specific site in bovine aortic endothelial cells

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Blood platelets have a major role in haemostasis through their aggregation and adherence to the damaged vessel wall. During this process, platelet granular constituents are released. Although the functions of many of these release products are well known, biological functions of some well defined platelet specific proteins have not been determined. This category includes platelet factor 4 (PF4) and β-thromboglobulin (βTG), both small proteins of molecular weight 30,000–36,000 (refs 1, 2) which are stored in the α granule of the platelet and have not been detected in any other tissue. βTG is composed of four identical noncovalently bound subunits and its amino acid sequence is significantly homologous with that of PF4 (ref. 3). Although PF4 possesses a marked ability to neutralise heparin, no physiological or pathophysiological role has been attributed to either protein. The discovery of prostacyclin (PGI_2) has led to a reappraisal of the factors regulating haemostasis and especially of the role of the vascular endothelium in preventing platelet adherence and aggregation[4–6]. The initial action of PGI_2 on the platelet is to stimulate adenylate cyclase[7–9], and it is the most potent inhibitor of platelet aggregation yet discovered. Also, cultures of arterial and venous endothelial cells have been found capable of producing PGI_2 (refs 10, 11). We now report results indicating that βTG reduces the production of PGI_2-like activity in cultured bovine endothelial cells, which are shown to possess a specific receptor for βTG. The characteristics of this receptor suggest that βTG may act locally at high concentrations to favour platelet aggregation by diminishing PGI_2 production.

Monolayer cultures of aortic endothelial cells were established from bovine aortas obtained from animals immediately after slaughter and processed within 1 h. Endothelial cells were prepared from the bovine aorta by collagenase digestion according to the method of Gimbrone *et al.*[12], and grown in plastic culture bottles in medium RPMI-1640 with 40% fetal calf serum. Small groups of cells became established, grew to near confluence within about 1 week and were subcultured by washing the monolayer surface with 0.02% EDTA followed by incubation in 0.025% trypsin–EDTA for 1–2 min. Complete culture medium (5 ml) was then added, and the cells were washed and resuspended in medium to be plated out in fresh vessels. All experiments were carried out on the first to the fourth subcultures. For studies of PGI_2 production, cells were suspended as in routine subculture, but were washed once more before resuspension in a buffered salt solution (BSS). Substances

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