Parotid secretion patterns during meals and their relationships to the tonicity of body fluids and to gastrin and pancreatic polypeptide in sheep

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The flow pattern of unilateral parotid saliva in sheep was compared when a total of 800 g lucerne (*Medicago sativa*) hay was offered as one, two, four or eight distinct meals. These patterns were related to changes in the tonicity of rumen fluid and plasma and to plasma concentrations of gastrin and pancreatic polypeptide. Sheep having *ad lib* access to hay overnight were offered fresh hay from 08·00 to 09·00 hours and were then given one, two, four or eight meals of fresh hay according to a schedule such that the mean deprivation period was 6·5 h for each meal frequency–size. Neither the peak in saliva flow rate nor the time of this peak differed among the different meal sizes. The flow rate decreased rapidly after reaching a maximum at 3·2 min into the meal. After 7 min of eating, the tonicity of plasma and rumen fluid had increased by only 2·2 and 8·2 mosmol/kg respectively. These increases would not cause the rapid decline in parotid flow observed after 3·2 min of eating. There was no postprandial change in the concentration of gastrin in jugular plasma. However, it did increase significantly ($P = 0·0043$) from 16 to 4 min before eating commenced. There was a postprandial peak in plasma pancreatic polypeptide concentration after 4·5 min of eating. However, the parotid flow rate remained low after the concentration of this peptide returned to prefeeding levels. The rapid decrease in parotid secretion rate observed early in the meal may be due to subsiding central excitation rather than to an inhibitory factor limiting production.

Gastrin: Pancreatic polypeptide: Saliva: Sheep

Parotid salivary secretion is markedly stimulated at the onset of eating, but declines despite the continuation of eating (Carr & Titchen, 1978). The mandibular secretion rate, however, remains elevated whilst eating continues (Carr, 1984). The marked stimulation and rapid decline in parotid salivary flow during a meal is unexplained and has been identified as an activity of the ruminant oral cavity requiring research (Reid, 1986). The rate of decline in parotid secretion during a meal is such that the secretion rate immediately after the end of the meal can be lower than during the period just before the start of the next meal, and this has been observed in both cattle (Bailey & Balch, 1961) and sheep (Wilson, 1963). Inhibition in salivary flow has been associated with an increase in osmolality of both rumen digesta (Warner & Stacy, 1977) and plasma (Carr & Titchen, 1978). Acute studies in sheep have also shown that pentagastrin acts centrally to inhibit parotid secretion (Grovum & Leek, 1988).

The purpose of the present study was to examine in greater detail the pattern of parotid flow during meals of different sizes given at different frequencies. The relationships between

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these flows and the tonicity of rumen digesta and plasma, as well as the plasma concentrations of gastrin and pancreatic polypeptide (PP), were examined. The peak in parotid saliva flow rate and the time of this peak did not differ with meal size. The flow rate decreased rapidly after reaching a peak at 3·2 min into the meal. It was concluded that the rapid decrease in parotid secretion was not caused by increases in the tonicity of rumen fluid or plasma, nor was it due to increases in the plasma concentration of gastrin or PP.

EXPERIMENTAL

Sheep and surgery

Four mature cross-bred Suffolk wethers prepared with rumen fistulas (Grovum, 1988) and reversible re-entrant unilateral parotid duct cannulas (Carter & Grovum, 1988) for collection of parotid saliva were used in the present study.

Housing and food

The sheep were held in metabolism crates in a temperature-controlled room with 24 h lighting. Fresh water was available at all times except when parotid saliva was being collected. Lucerne (*Medicago sativa*) hay was available *ad lib* except during experiments. The hay contained (g/kg) 890 dry matter, 236 crude protein (nitrogen × 6·25), 19 fat, 65 ash and 412 acid-detergent fibre. The sheep were accustomed to the metabolism crates and hay before the start of the experiment.

Experimental procedure

On an experimental day all sheep were offered one meal of 800 g, two meals of 400 g, four meals of 200 g or eight meals of 100 g. It was necessary that all sheep received the same meal frequency–size on an experimental day, as parotid secretion can be stimulated in sheep when they observe other sheep being fed, or recognize events associated with feeding (Denton, 1957). Sheep had *ad lib*. access to hay overnight and were offered fresh hay from 08.00 to 09.00 hours. They were then fasted with meal times arranged such that the mean deprivation period was 6·5 h for each meal frequency–size (i.e. one meal of 800 g was given at 15.30 hours; two meals of 400 g were given at 14.30 and 16.30 hours; four meals of 200 g were given at 12.00, 14.20, 16.40 and 19.00 hours; eight meals of 100 g were given hourly from 12.00 to 19.00 hours). It was not possible to record the parotid flow rate and sample blood and rumen contents from all sheep simultaneously. However, recording and sampling for any particular meal size was replicated two, three or four times in each sheep. Drinking water was removed during recording and sampling. The end of a meal was assessed by the time-period spent idling and was set at 3.0, 2.0, 1.5 and 1.0 min for 800, 400, 200 and 100 g meals respectively.

The parotid salivary flow was recorded before (15 min) and during eating. Secretion was recorded by attaching a fluid-filled tube to the side-arm of the stopcock on the re-entrant cannula and leading this tube from the lumbar region of the sheep's back down the side of the cage into a measuring cylinder. The tip of the tube was located just below the level of the feeder to create slight negative pressure on the parotid duct during eating and, thus, prevent saliva leakage around the cannula. Blood from a jugular catheter and rumen fluid samples from a probe with the tip located in the ventral sac were taken 4 and 16 min before feeding. Recordings of saliva volume and samples of rumen digesta (approximately 10 ml) and blood (10 ml) were taken as frequently as possible at the start of eating (every 1–3 min) to determine changes associated with the peak in parotid flow. Blood samples (heparinized) were divided for determination of osmolality and for analysis of gastrin and PP and placed on ice immediately after collection. Samples for peptide analysis were treated with 500 KIU

(Kallikrein inactivating units) aprotinin (Trasylol; Miles Pharmaceuticals, Rexdale, Ontario M9W 1G6, Canada)/ml blood to inhibit proteinase activity. They were centrifuged the same day to obtain plasma which was stored at $-20°$ until analysis. Gastrin and PP concentrations were determined by a radioimmunoassay technique (Hall *et al.* 1983). The antibody used for gastrin analysis reacted with all molecular forms of gastrin. Osmolality was determined from freezing-point depression with a digimatic osmometer model 3DII (Advanced Instruments Inc., Needham Heights, Mass., USA).

Statistical analysis

Analysis of variance was applied to the mean responses of the sheep to the four meal sizes treated as a randomized complete block design. There was one missing set of values for the 400 g meal size and so the adjusted treatment means were compared with the protected least significant difference test. The changes in osmolality of plasma and rumen fluid, and the concentrations of plasma gastrin and PP, from before eating to times just after the peak of parotid flow were pooled for all sheep offered all meals. The differences in the respective values before and during feeding were analysed by Student's paired *t* tests to determine if the average changes were greater than zero (differences accepted as real when $P < 0.05$).

RESULTS

Findings obtained from the present study are summarized in Table 1. The sheep consumed approximately half the amount of food offered for reasons that could not be determined. This was more evident with the larger meal sizes when eating behaviour may have been affected by the collection of unilateral parotid saliva. However, food intake did increase progressively when 100–800 g was offered, but there was a fivefold, rather than the intended eightfold difference in meal size. The meal duration increased as the amount consumed increased. There were no differences in the premeal or peak parotid flow rates between the meal sizes. However, unilateral parotid flow rates were markedly stimulated at the onset of eating. The increase, pooled across meal sizes and sheep, was from a mean of 1·6 (SE 0·16) ml/min to 6·4 (SE 0·47) ml/min. There was no significant difference in the times at which the peak flows occurred, the mean for all treatments being 3·2 min.

The premeal tonicity of plasma was 293·6 mosmol/kg. The increment in tonicity of plasma from 4 min before eating to 7 min after the start of eating was not significantly different among meal sizes. The pooled mean increase of 2·2 (SE 0·7) mosmol/kg was significantly greater than zero ($P = 0·0036$). The pooled mean increment in plasma tonicity 12 min after initiation of eating was 3·0 (SE 0·82) mosmol/kg which was also greater than zero ($P = 0·0013$). The mean increases in tonicity of rumen fluid from a control value of 265·2 (SE 5·2) mosmol/kg at 3 min before feeding to 7 and 12 min after the start of eating were 8·2 (SE 2·6) and 18·5 (SE 3·9) mosmol/kg respectively. Both these increases were significantly greater than zero ($P = 0·0053$ and $P = 0·0001$ respectively). There was also a difference between meals for the increase after 7 min of eating with the two largest meals resulting in the greatest increases.

The changes in plasma concentration of gastrin and PP from 4 min before feeding to 7 and 12 min after the start of eating were not different among meal sizes. The pooled mean gastrin concentrations were 33 (SE 1·8), 33 (SE 2·0) and 32 (SE 1.8) pmol/l plasma for the three times respectively. No postprandial increase in plasma gastrin concentration was observed for any meals. However the concentration of gastrin increased from 16 to 4 min before offering food in nineteen of twenty-seven meals and resulted in a significant rise of 4 pmol/l ($P = 0·0043$).

The pooled mean PP concentrations at -16, $-4·0$, 4·5, 7·0, 12, 24, 34 and 43 min were

Table 1. *Effect of meal size on dry matter intake, meal duration, unilateral parotid flow pattern, changes in osmolality of plasma and rumen fluid, and changes in plasma gastrin and pancreatic polypeptide (PP) concentrations in sheep*

	Meal size (g air-dry wt)				Statistical significance	ASED
	100	200	400	800		
Dry matter intake (g)	69·1[a]	116·2[a, c]	225·6[b, c]	328·5[b]	**	61·6
Meal duration (min)	10·25[a]	18·7[a]	34·0[b]	44·1[b]	***	5·63
Premeal† (15 min) parotid flow (ml/min)	1·32	1·75	1·98	1·31	NS	0·55
Meal peak parotid flow rate (ml/min)	7·32	5·90	7·01	6·97	NS	1·14
Time of peak flow (min)	1·25	4·12	3·64	3·47	NS	1·09
Δ Plasma osmolality peripeak–premeal (mosmol/kg)	3·02	0·72	1·24	4·79	NS	3·12
Δ Rumen osmolality peripeak–premeal (mosmol/kg)	1·14[a]	3·39[a]	18·6[b]	23·5[b]	**	4·35
Premeal gastrin concentration (pmol/l plasma)	34·5	36·2	31·1	30·7	NS	4·67
Peripeak gastrin concentration (pmol/l plasma)	32·5	36·7	28·7	31·8	NS	4·42
Postpeak gastrin concentration (pmol/l plasma)	34·6	34·7	25·5	28·2	NS	3·43
Premeal PP concentration (pmol/l plasma)	139·5[a]	236·7[b]	210·0[a, b]	252·4[b]	*	42·2
Peripeak PP concentration (pmol/l plasma)	145·0[a]	270·9[b]	212·2[a, b]	294·9[b]	*	43·4
Postpeak PP concentration (pmol/l plasma)	149·0	236·4	200·6	267·4	NS	38·4

[a, b, c] Means in a row with different superscript letters were significantly different (protected least significant difference test; $P < 0.05$). NS, not significant; ASED, average standard error of difference over the six possible differences between the four means.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Premeal is the sample just before feeding, peripeak and postpeak are the samples nearest the peak parotid flow rate and the next sample respectively.

190, 208, 240, 223, 204, 173, 192 and 200 pmol/l respectively. Thus, there was a postprandial peak in PP after 4·5 min of eating when a concentration of 240 (SE 23·6) pmol/l was attained. Analysing the paired differences showed that this was an increase of 47 ($P = 0.0005$) and 27 pmol/l ($P = 0.002$) compared with the respective concentrations measured 16 and 4 min before offering hay.

The unilateral parotid flow patterns obtained in two sheep are shown in Fig. 1. These illustrate that the peak flow was attained rapidly after the onset of eating, and that the decline in parotid flow rate occurred as eating continued. Differences among animals were apparent with respect to the value of the peak flow and the duration of the elevated flow. The peak flow was not correlated with the amount of food offered or consumed (Table 1). The parotid salivary flow rate in a sheep which was offered three small meals (approximately 100 g) in succession, allowing each meal to be completed before offering the next meal, is shown in Fig. 2.
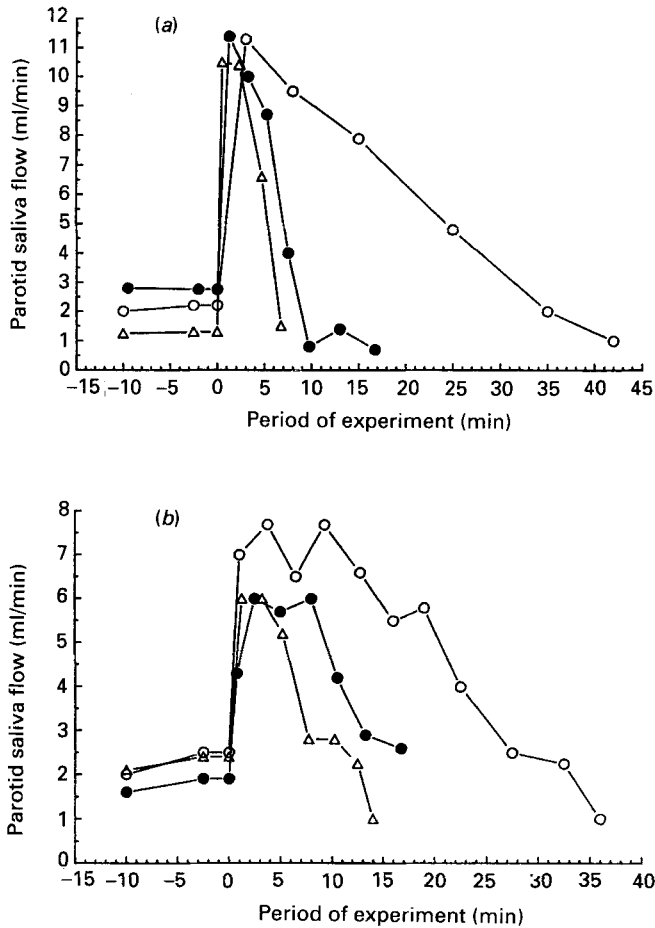
Fig. 1. Unilateral parotid salivary flow in response to consumption of lucerne (*Medicago sativa*) hay given at time 0 in sheep no. 1 ((*a*): (△), 84 g dry matter (DM); (●), 151 g DM; (○), 484 g DM) and in sheep no. 2 ((*b*); (△), 69 g DM; (●) 84 g DM; (○), 217 g DM). Meal termination corresponds to the end of the lines.

DISCUSSION

Carr & Titchen (1978) recorded parotid saliva volume over 5 min intervals and showed that the peak flow occurred 10 min after 1 kg lucerne chaff was offered. They reported that the elevated flow rate was maintained for 15–20 min. The shorter sampling intervals used in the present study showed that the peak flow occurred within 4 min of the animals commencing meals of lucerne hay. In meals of 69·1 g lasting 10·25 min, the peak flow was after 1·25 min of eating. Although the rate of food consumption and the frequency of chewing were not measured, it was clear that the parotid flow rate declined despite the animals continuing to eat avidly. Whilst the side of the mouth engaged in chewing was not recorded, it has been assumed that a change from the side of parotid collection to the contralateral side did not occur within 5 min of the sheep starting to eat. Carr & Titchen (1978) reported that the flow rate declined after 15–20 min if the animals continued to eat, but the eating rate is more likely to have decreased after this period than 1·25 min after starting meals lasting 10·25 min.
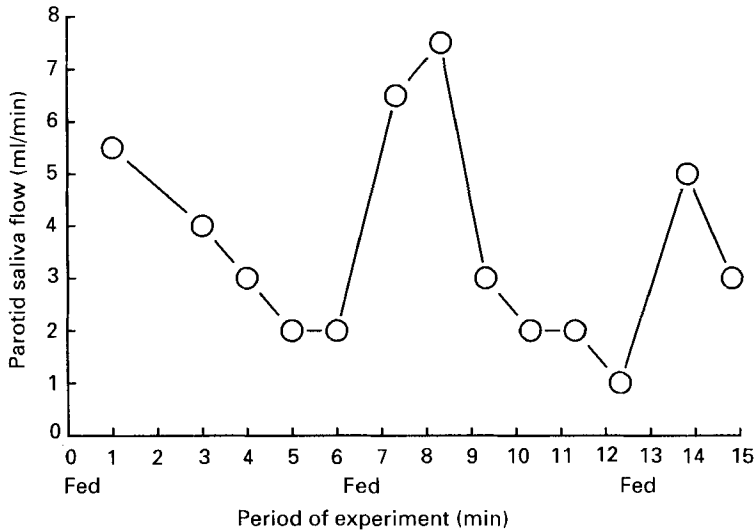
Fig. 2. Unilateral parotid salivary flow from sheep no. 1 in response to three successive meals of lucerne (*Medicago sativa*) hay (approximately 100 g each). Each meal was consumed before the next one was offered.

Warner & Stacy (1977) demonstrated a negative association between osmolality of rumen fluid and the rate of secretion of mixed saliva collected from oesophageal fistulas. Increasing the osmolality of rumen fluid from 210 to 440 mosmol/kg did not, however, inhibit parotid salivary flow in anaesthetized sheep (Carter *et al.* 1985). Thus, while there was a small but significant rise of 8·2 mosmol/kg in rumen fluid tonicity in association with the decline in parotid flow, a causative relationship is unlikely. Furthermore, water was removed during meal consumption, thus preventing drinking which would normally occur during a meal.

Increasing the tonicity of jugular plasma by 15–16 mosmol/kg inhibited unilateral parotid flow by approximately half in conscious sheep (Warner & Stacy, 1977). In anaesthetized sheep the tonicity of carotid arterial plasma had to be raised by 7–12 mosmol/kg before a detectable inhibition in parotid saliva flow was observed (Carter *et al.* 1985). In the present study, the peak in parotid flow occurred after 3·2 min of feeding, whereas the tonicity of jugular plasma increased by only 2·2 mosmol/kg after 7 min of eating. Hence, this small increase in plasma tonicity could not be responsible for the dramatic reduction in parotid flow after the peak.

A rise in systemic gastrin was observed when a single daily meal of 800 g lucerne pellets was offered to sheep weighing 31–40 kg (Reynolds *et al.* 1978). In the present study, sheep offered one meal of 800 g lucerne hay were deprived of food for only 6·0–6·5 h and no postprandial rise was observed in plasma gastrin concentration. Similarly, there was no postprandial rise in plasma gastrin when smaller meals were offered. Thus, the absence of a postprandial increase may be due to an insufficient fasting period to achieve true basal levels. Whilst pentagastrin can inhibit parotid secretion (Grovum & Leek, 1988), there was no increase in systemic gastrin levels associated with the decline in parotid flow during meals in the present experiments. This does not eliminate the possibility that a rise in gastrin concentration elsewhere in the animal may be associated with the rapid decline in parotid flow. Gastrin-like immunoreactivity has been found in the central nervous system, primarily in the hypothalamus, infundibulum, pituitary and medulla (Rehfeld, 1978).

However, it is not known if this gastrin is released postprandially to act in the brain, or if it would inhibit parotid secretion if it were released.

Pancreatic polypeptide has not been considered to be involved in the salivary response during feeding, but was measured to ascertain whether a postprandial response occurred with the feeding regimen adopted in the present study. In sheep fed *ad lib*, serum PP did not increase postprandially, but rather fell over a 4 h period after the provision of fresh food despite avid food consumption for at least the first 60 min (Hansky *et al*. 1980). However, the first blood sample after feeding was at 30 min from the start of the meal. Pooling all observations in the present study resulted in a mean increase in plasma concentration of this peptide of 15 pmol/l from 4 min before to 7 min after the start of the meals. This was followed by a significant decrease of 19 pmol/l 5 min later to a level below control values (204 *v*. 208 pmol/l; not significant). This supports the trend observed by Hansky *et al*. (1980), but their blood sampling frequency did not detect the small postprandial peak observed at 4·5 min in the present experiments. This pattern is similar to that reported in calves where a transient postprandial increase in PP occurred within 30 min and was followed by a slight decrease to levels below basal (Bloom *et al*. 1978).

The peaks (pooled across meals and sheep) in plasma concentration of PP and parotid salivary flow were close (4·5 and 3·2 min respectively) but the parotid flow rate remained low after the PP concentration had decreased. This suggests that this peptide was probably not involved in producing the observed pattern of parotid flow. It is possible that another peptide or hormone, other than those measured, may have been responsible for the rapid decline in parotid flow. However, it seems unlikely as parotid flow was re-stimulated when a second meal was offered immediately after the first (Fig. 2).

The rise in PP from 16 min before eating to 4·5 min after feeding and the increase in gastrin from 16 to 4 min before eating may represent a cephalic, vagally stimulated, secretion. This argument is supported by the finding that the postprandial increase in PP secretion in suckled calves was blocked by atropine (Bloom *et al*. 1978). Shulkes & Hardy (1980) also used atropine to block the increase in PP secretion induced by bethanecol or bombesin in sheep. These findings indicate that PP release is at least partly under vagal cholinergic control. Cephalic–vagal stimulation of PP secretion has been demonstrated in man (Taylor *et al*. 1978). Insulin injected intravenously led to an increase in serum gastrin from 38 (SE 0·6) to 68 (SE 1·0) pmol/l in three sheep, an effect interpreted as vagally stimulated secretion (Reynolds *et al*. 1979) because insulin-induced gastrin release requires intact vagal fibres (Hansky *et al*. 1972). Both volume and concentration of pepsin and acid from abomasal pouches were increased 15–30 min after sheep were teased with food or fed (McLeay & Titchen, 1970). This was interpreted as a cephalic phase of gastric secretion which was probably at least in part the result of vagally induced gastrin release. The prefeeding increase in concentrations of gastrin and PP obtained in the present experiments supports the evidence for a cephalic phase of digestion in the sheep. However, mixing contractions of the reticulo-rumen also increase in response to teasing with food, which would lead to increased digesta flow to the abomasum. Chemical stimuli could then stimulate gastrin release. Hill (1965) contended that the increased abomasal secretion after insulin-induced hypoglycaemia was primarily due to the increased flow of digesta from the reticulo-rumen as a result of insulin-induced hypermotility, rather than from vagal stimulation. Similarly, Taylor *et al*. (1978) found a more marked increase in serum PP concentration when homogenized food was infused directly into the stomachs of human subjects than when an appetizing meal was chewed and expectorated, hence producing cephalic–vagal stimulation. The rapid decline in parotid salivary flow that occurred within 5 min of sheep starting a meal could not be accounted for by changes in the osmolality of rumen fluid or plasma, or changes in the plasma concentration of gastrin and PP, or in the

eating rate, because the animals continued to eat avidly during this period. Psychic or cephalic stimulation of parotid secretion was reported in sheep with stimuli associated with feeding (Denton, 1957). The duration of the parotid response was as long as 55 min, suggesting it was due to parasympathetic stimulation rather than to expulsion of preformed saliva caused by increased sympathetic activity. Continual stimulation of the parotid nerve in acute experiments with sheep results in parotid secretion for considerably longer than 3·2 min (R. R. Carter and W. L. Grovum, unpublished results). Whilst tachyphylaxis has been shown to occur in the rat parotid gland with prolonged parasympathetic stimulation (Ekstrom et al. 1985), it would not explain the decline in parotid flow observed in the current study as secretion could be re-stimulated when a new meal was presented to the sheep as shown in Fig. 2.

Blood flow to the parotid gland in sheep increased from 169 ml/min per 100 g tissue at 20–30 min before eating to 365 ml/min per 100 g tissue after 3–4 min of feeding (Barnes et al. 1983). This was attributed to parasympathetic vasodilatation. After 2 h, the blood flow had returned to 127 ml/min per 100 g tissue. Blood flow through the gland is a determinant of the rate of secretion (Coats et al. 1956). It is, therefore, possible that central excitation at the onset of eating increased parasympathetic activity to the parotid glands and, hence, increased both secretion and blood flow. Excitation associated with presentation of food is suggested by the increases in salivation (Fig. 2) when new meals were offered immediately after the completion of the previous meal. Furthermore, when eight meals of 100 g were offered daily at hourly intervals, the peak flow did not differ between earlier and later meals (R. R. Carter, unpublished results). Taste and mechanical stimulation in the mouth would presumably stimulate parotid secretion throughout the meal. The rapid decline in secretion early in the meal may be due to a subsiding central excitation rather than to an inhibitory factor.

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