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# Contractile effects of substance P and neurokinin A on the rat stomach *in vivo* and *in vitro*

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**1** Substance P and neurokinin A (substance K) were infused into the coeliac artery of anaesthetized rats at doses of 0.06–20 nmol $min^{-1}$. Both tachykinins caused contractions of the stomach, the threshold dose of neurokinin A being 10 times lower than of substance P. The dose-response curve for substance P was flatter than that for neurokinin A.

**2** On circular muscle strips from the rat gastric corpus *in vitro*, the dose-response curves for both tachykinins were parallel, neurokinin A being 10 times more potent than substance P. The contractions in response to 10 μM neurokinin A and to 30 μM substance P were 58 and 54%, respectively, of the maximal contraction to bethanechol (1 mM).

**3** The effect of substance P was reduced by atropine both *in vivo* and *in vitro*. *In vitro*, the contractions to substance P were also reduced by tetrodotoxin but left unaffected by methysergide. The action of neurokinin A was not affected by these drugs.

**4** It is concluded that neurokinin A contracts rat stomach by a direct action on the circular smooth muscle, whereas the action of substance P is mediated, at least in part, by cholinergic interneurones.

## Introduction

Substance P (SP) has previously been shown to contract the stomach of various animals in a variety of preparations both *in vivo* and *in vitro* (Bertaccini & Coruzzi, 1977; Milenov *et al.*, 1978a,b; Edin *et al.*, 1980; Milenov & Golenhofen, 1983). In the rat the stomach is innervated by SP-immunoreactive nerves (Schultzberg *et al.*, 1980) which are of both extrinsic (sensory) and intrinsic origin (Minagawa *et al.*, 1984; Sharkey *et al.*, 1984; Ekblad *et al.*, 1985). Neurokinin A-like immunoreactivity has a distribution pattern similar to SP (Theodorsson-Norheim *et al.*, 1984), and binding sites for both SP and neurokinin A (substance K, NKA) are present in high numbers throughout the gastric smooth muscle layers, particularly in the circular muscle (Burcher *et al.*, 1986).

So far, two different preprotachykinin mRNAs encoding NKA have been identified in mammals (Nawa *et al.*, 1984b; Krause *et al.*, 1987), both of which also encode SP. Thus it is highly probable that, wherever NKA occurs, it coexists with SP. Consequently, SP and NKA might act on the same postsynaptic structures or, at least, influence each other in this action. SP and NKA have been previously found to affect potently gastric emptying in the rat; emptying seemed to depend on whether contraction of the stomach or pylorus prevailed (Holzer, 1985). The precise nature of the effects of SP and NKA on gastric contractility is, however, not evident from these data. We therefore investigated the motor effects of the two tachykinins on gastric circular muscle and some of their pharmacological characteristics in more detail. The effects were tested both *in vitro* and *in vivo* in view of the finding that, in the dog gut, the effects of SP differ when examined on isolated preparations or in the living animal (Fox *et al.*, 1983).

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## Methods

### *Animals*

Sprague–Dawley rats (Institut für Versuchstierkunde, Himberg, Austria) of either sex weighing 250–300 g (females) and 300–400 g (males) were used. Before the experiments, the rats were fasted over night.

In vivo *experiments*

The rats were anaesthetized with urethane (1.25 g $kg^{-1}$ i.p.). The trachea was cannulated, and blood pressure was monitored from a carotid artery. The oesophagus was ligated in the neck. The abdominal aorta was exposed through a midline laparotomy and a PE-20 cannula was introduced into the aorta in a retrograde direction until the tip of the cannula lay at the branching of the coeliac artery. This cannula was connected to an infusion pump and Tyrode solution was continuously infused at a rate of 60 $\mu l\,min^{-1}$. When the peptides were to be tested, the infusion was switched to the appropriate solution for 5 min. At least 15 min were allowed between two consecutive test infusions. By means of an infusion of Evans blue it was proved that such an intra-aortic infusion reached the upper gastrointestinal tract from the corpus of the stomach to the first 4–5 cm of the duodenum but spared the gastric fundus.

Changes in intragastric pressure were recorded through a catheter introduced into the stomach via the duodenum and fixed by a ligation around the pylorus. By means of a Y-shaped adapter, this catheter was connected to a reservoir of Tyrode solution and to a Statham pressure transducer. Fifteen min after the end of the operation, 3 ml Tyrode solution (2 ml for rats below 300 g body weight) was rapidly infused into the stomach.

For guanethidine pretreatment the rats received 10 mg $kg^{-1}$ of the compound subcutaneously in the evening, and 10 mg $kg^{-1}$ in the morning before the experiment.

In vitro *experiments*

The rats were killed by a blow on the head. The stomach was rapidly taken out and circular strips were cut from the gastric corpus. The mucosa was dissected off, and the muscular strips, 2 mm wide and 10–12 mm long, were suspended in a 3 ml organ bath containing Krebs solution at 37°C gassed with 5% $CO_2$ and 95% $O_2$ (composition in mM: NaCl 118.0, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5, $KH_2PO_4$ 1.18, $NaHCO_3$ 25.0, glucose 11.1). Isometric contractions were measured by a force displacement transducer (Hugo Sachs Elektronik, Freiburg, FRG). After an equilibration period of 45 min, the tissue was stimulated 2–3 times with a maximally effective dose of bethanechol (1 mM). Then, conventional dose-response curves were constructed. The tissue was washed as soon as the peak contraction had developed, which took up to 45 s for bethanechol, up to 3 min for SP, and up to 4 min for NKA.

When tetrodotoxin (TTX, 1 $\mu$M) was tested, it was added to the organ bath 30 s before each addition of agonist. Atropine (0.6 $\mu$M) or methysergide (0.1 $\mu$M) was added to the Krebs solution reservoir. Since a decrease in tissue sensitivity with time was observed, the series of agonists were added twice, TTX or one of the antagonists being added after the 2nd series of agonist. For control experiments, two identical series of agonists were tested on one strip. For evaluation of these experiments, the change in contractile force between the first and second addition of a particular dose of agonist on the same strip was calculated (as the difference between the first and second contraction). If

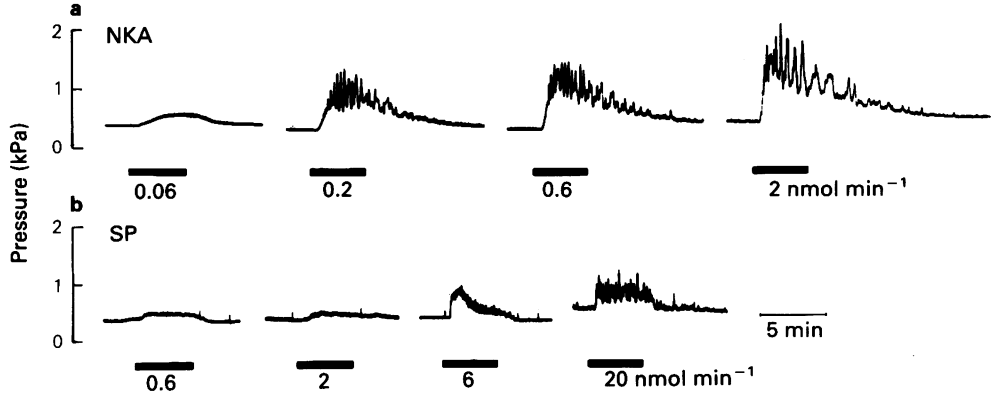


**Figure 1** Typical tracing of the rat stomach *in vivo* contracting in response to infusions of (a) neurokinin A (NKA) or (b) substance P (SP) into the coeliac artery as indicated by the bars.

one of the antagonists added was to reduce the response to the agonist, the difference would be expected to be greater than in control strips without antagonist.

*Substances*

SP and NKA were obtained from Cambridge Research Biochemicals (Cambridge, UK), bethanechol-Cl from Schuchardt (München, FRG), atropine sulphate from Merck (Darmstadt, FRG), guanethidine from CIBA-Geigy (Basle, Switzerland), dimethylphenylpiperazinium-iodide (DMPP) and TTX from Sigma (Deisenhofen, FRG), 5-hydroxytryptamine creatinine sulphate from Fluka (Buchs, Switzerland), and methysergide maleate from Sandoz (Basle, Switzerland). Stock solutions (1 mM) of the peptides were made with 0.01 M acetic acid, of the other substances with 0.15 M NaCl. Dilutions for *in vivo* use were made with Tyrode solution, for *in vitro* use with isotonic saline.

## Results

In vivo *experiments*

After filling the stomach with a volume of 2 or 3 ml Tyrode solution, intragastric pressure equilibrated at 0.1–0.3 kPa. No spontaneous phasic contractions occurred with this tone. Infusion of 0.6–20 nmol $min^{-1}$ SP or 0.06–2 nmol $min^{-1}$ NKA into the coeliac artery for 5 min led to dose-dependent tonic contractions of the stomach; the higher doses used also caused phasic contractions (Figures 1 and 2). The threshold dose of SP was somewhat more than 10 times higher than that of NKA. The dose-response curve for SP appeared flatter than that for NKA and, in the dose range used, the strongest effects produced by SP were smaller than those produced by NKA.

The two higher doses of SP (6 or 20 nmol $min^{-1}$) led to short decreases in blood pressure by 0.6–2.6 kPa, whereas hardly any hypotension was observed during an infusion of NKA.

Pretreatment of the rats with guanethidine (20 mg $kg^{-1}$ s.c.) shifted the dose-response curves for both tachykinins to the right without changing their relative positions (Figure 2).

Atropine (1 mg $kg^{-1}$ i.v.) reduced basal intragastric pressure if it was higher than 0.4 kPa at the time of injection. Atropine did not influence the effect of a test dose of NKA (0.6 nmol $min^{-1}$) (Figure 3). In contrast, the tonic contractions in response to 6 nmol $min^{-1}$ SP were significantly reduced by atropine, and phasic contractions did not occur in 4 of 6 preparations. As expected, the contractions due to bethanechol (60 nmol $min^{-1}$) were completely abolished by atropine.

In vitro *experiments*

Circular muscle strips of the gastric corpus responded to a maximally effective dose of bethanechol (1 mM) with a mean force development of 58 ± 6 mN ($n$ = 5). NKA and SP were less efficacious: 30 μM SP induced a maximal effect only 54 ± 4% ($n$ = 6), and 10 μM NKA

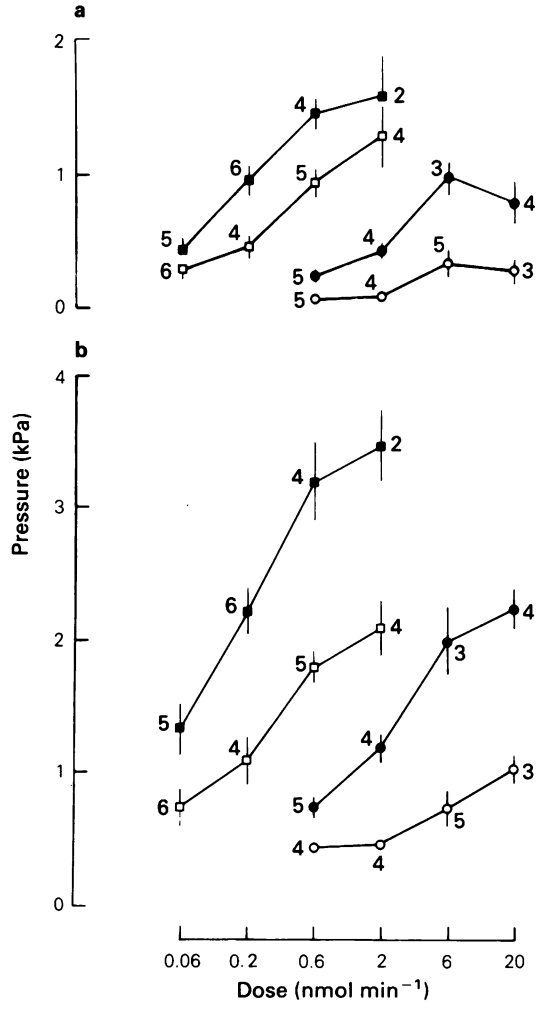


**Figure 2** Dose-response curves for the contractile effects of substance P (SP, ○, ●) and neurokinin A (NKA, □, ■) on the rat stomach *in vivo* during infusion into the coeliac artery. (a) Increase in baseline pressure (tonic contraction). (b) Maximum pressure at the peak of the phasic contractions (○, □) Untreated rats; (●, ■) guanethidine-pretreated rats. Each point represents the mean and vertical lines show s.e.mean. $n$ indicated by the number beside each point.

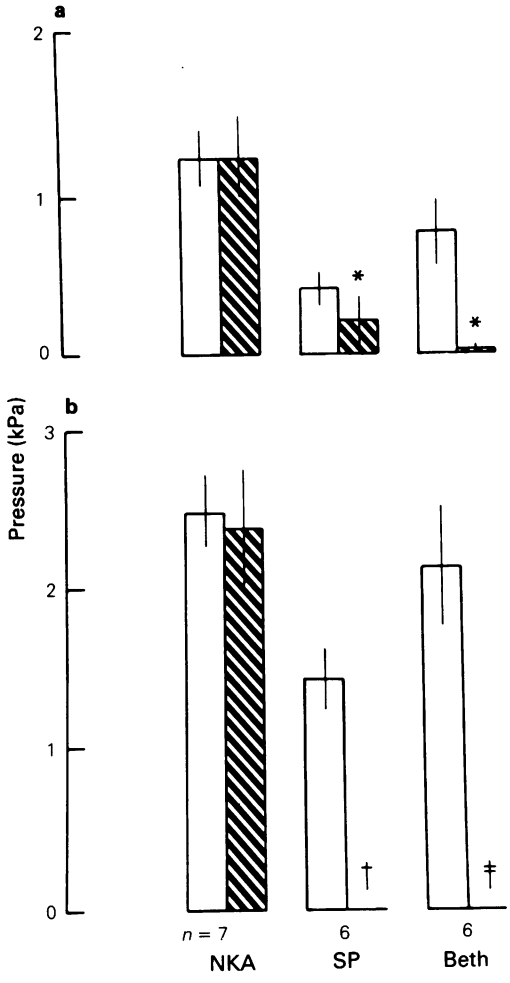


**Figure 3** Effect of atropine (1 mg kg$^{-1}$, i.v.) on gastric contractions due to 0.6 nmol min$^{-1}$ neurokinin A (NKA), 6 nmol min$^{-1}$ substance P (SP) and to 60 nmol min$^{-1}$ bethanechol (Beth). (a) Increase in baseline pressure (tonic contraction). (b) Maximum pressure at the peak of the phasic contractions. Open columns represent mean responses before atropine and hatched columns represent mean responses after atropine. Vertical lines show s.e.mean. † No phasic contractions in 4 of 6 experiments. ‡ No phasic contractions. *$P < 0.05$ vs control.

induced one only 58 ± 4% ($n = 7$), of the maximal response to bethanechol (Figure 4).

The contractions elicited by NKA were not affected by either TTX, atropine or methysergide (Tables 1 and 2). The doses of antagonists used were sufficient to reduce significantly the effect of DMPP (100 μM) or

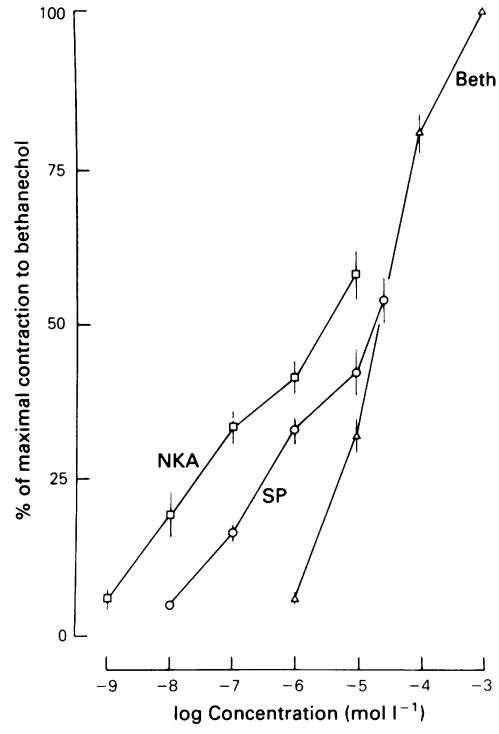


**Figure 4** Dose-response curves for the effects of substance P (SP, ○), neurokinin A (NKA, □) and bethanechol (Beth, Δ) on the isolated circular muscle strips from the rat gastric corpus *in vitro*. Each point represents the mean, $n = 5$–7, and vertical lines show s.e.mean.

bethanechol (10 μM or 1 mM), and to abolish the action of 5-hydroxytryptamine (10 μM), respectively. The contractions induced by SP were, however, reduced by TTX, although this decrease did not reach statistical significance for the highest dose of SP examined (10 μM) (Table 1). The effect of this dose was significantly reduced by atropine. No effect of methysergide was observed (Table 2).

## Discussion

The present results provide evidence that NKA, as well as SP, is able to contract the stomach of the rat, NKA being ten times more potent than SP. However, there are differences in the mode of action of the two tachykinins: NKA appears to act directly on gastric circular smooth muscle, as it is not inhibited by TTX. In contrast the effect of SP seems to be mediated, at least in part, by cholinergic excitatory interneurones

**Table 1** Influence of tetrodotoxin (TTX, 1 μM) on the contractile effect of several agonists on circular muscle strips of the rat gastric corpus

| | *Control* | *TTX* |
|---|---|---|
| NKA 1 nM | −8.4 ± 2.7 | −15.8 ± 3.3 |
| 10 nM | −2.7 ± 1.6 | −8.0 ± 3.9 |
| 100 nM | −1.2 ± 1.8 | −3.1 ± 4.3 |
| 1 μM | −0.7 ± 3.1 | −4.9 ± 3.6 |
| 10 μM | −1.2 ± 1.4 | −6.5 ± 4.8 |
| SP 10 nM | −1.4 ± 0.9 | −5.6 ± 1.4* |
| 100 nM | −1.2 ± 0.7 | −10.3 ± 2.4** |
| 1 μM | −0.4 ± 0.5 | −16.5 ± 7.0* |
| 10 μM | −2.2 ± 2.4 | −6.7 ± 11.5 |
| DMPP 100 μM | −3.5 ± 3.0 | −14.8 ± 4.7** |
| Beth 1 mM | −11.1 ± 2.9 | −8.3 ± 3.9 |

The values (mean ± s.e.mean, $n = 5–10$) denote the difference in contractile force (in mN) between the 1st and 2nd addition of a particular dose; i.e. a negative value means a decrease in sensitivity after th 2nd addition of the agonist, a positive value means an increase in sensitivity; a value of 0 denotes the same response after both additions of the agonist (for details see Methods).
NKA = neurokinin A; SP = substance P; DMPP = dimethyl-phenylpiperaziniumiodide; Beth = bethanechol.
*$P < 0.05$, **$P < 0.01$ vs control (one-sided Mann-Whitney U-test).

since it is antagonized by atropine, this antagonism being more pronounced *in vivo* than *in vitro*. This observation is similar to one described by Fox *et al.* (1983) for the dog, namely indirect, nerve-mediated effects being more pronounced *in vivo* than *in vitro*. 5-Hydroxytryptaminergic interneurones did not seem to play a role in mediating the effect of SP. In contrast to the present observations, Lidberg *et al.* (1985) described a reduction of the action of SP on pyloric and antral longitudinal strips by $10^{-8}$–$10^{-5}$ M ketanserin. In our study, however, 100 nM ketanserin had no consistent effect on the response of either longitudinal fundus or circular corpus strips to 10 or 100 μM 5-hydroxytryptamine (6 observations), which is supported by the findings of Van Nueten *et al.* (1984) that ketanserin does not affect the motility of gastrointestinal tissues *in vitro*.

SP and NKA are 10 and 100 times, respectively, more potent than bethanechol in contracting the rat stomach, although the maximal contraction due to bethanechol *in vitro* is almost double that due to the tachykinins. It was not possible to test dose-response relationships for muscarinic agonists on the stomach *in vivo* since acetylcholine itself, possibly due to its fast metabolism, did not elicit any gastric contractions even after an infusion of extremely high doses. Also, if doses of bethanechol higher than 60 nmol $min^{-1}$ were tested, they elicited intense systemic effects which would have rendered the gastric recordings unreliable.

A striking observation was that, contrary to the *in vitro* experiments, the dose-response curves for SP *in vivo* were flatter than those for NKA. This result might be explained in several ways: firstly, it was observed that SP, being more potent than NKA in reducing blood pressure (Nawa *et al.*, 1984a), also caused a short-lasting hypotension. Therefore, it might have led to a reflex increase in sympathetic nerve activity, which, in turn, would have reduced the contractility of gastric smooth muscle. This possibility was examined by repeating the experiments in rats pretreated with guanethidine. In these animals, however, instead of approximating the dose-response curve for SP to that of NKA, the effects of both SP and NKA were markedly increased without change in the difference in potency between the two tachykinins. This can be explained by the observation that laparotomy as such is known to cause a massive increase in activity of the sympathetic nervous system with concomitant inhibi-

**Table 2** Influence of atropine (0.6 μM) and methysergide (0.1 μM) on the contractile effect of neurokinin A (NKA), substance P (SP) and bethanechol (Beth) on circular muscle strips of the rat gastric corpus

| | | *Control* | *Atropine* | *Methysergide* |
|---|---|---|---|---|
| NKA | 100 nM | −0.6 ± 0.3 | −2.4 ± 0.9 | −0.8 ± 0.2 |
| | 10 μM | +0.1 ± 0.2 | −0.8 ± 0.4 | 0.0 ± 0.6 |
| SP | 1 μM | −1.9 ± 0.5 | −2.5 ± 0.6 | −1.5 ± 0.2 |
| | 10 μM | −0.6 ± 0.5 | −3.2 ± 0.6** | −0.9 ± 0.4 |
| Beth | 10 μM | +0.1 ± 0.2 | −6.2 ± 1.8* | — |
| | 1 mM | 0.0 ± 0.3 | −4.5 ± 1.7** | — |

The values (mean ± s.e.mean, $n = 6–7$) denote the difference in contractile force (in mN) between the 1st and 2nd addition of a particular dose (for further details see Table 1 and Methods).
*$P < 0.05$, **$P < 0.01$ vs control (one-sided Mann-Whitney U-test).

tion of gastrointestinal motility (Furness & Costa, 1974). Thus, guanethidine appears simply to have abolished the effects of laparotomy.

A second reason for the smaller efficacy of SP compared with NKA *in vivo* might be because it is metabolized by enzymes which do not cleave NKA. This view is supported by the observation of Holzer (1985) that the hypotensive effect of NKA after intraperitoneal administration lasts longer than that of SP. Acetylcholinesterase has been shown to hydrolyse SP (Chubb *et al.*, 1980). Whether it fails to hydrolyse NKA has not been examined, but this possibility is not totally improbable, since it was demonstrated recently that angiotensin converting enzyme cleaves SP but not NKA (Turner & Hooper, 1987). In contrast, in a study on rabbit blood pressure and rat salivation *in vivo*, after intravenous injections of the tachykinins, no differences in the slope of the dose-response curves were observed (Holzer-Petsche *et al.*, 1985). In fact, the results indicated, if anything, that NKA was the tachykinin being metabolized faster than the other.

A third explanation might come from the finding of Burcher *et al.* (1986) that the number of binding sites for NKA in the rat stomach is greater than that of SP. This could be a possible basis for the greater efficacy of NKA compared to SP, although it is not clear why this should determine the action of the tachykinins only *in vivo* but not *in vitro*.

Fourthly, one major difference between the preparations *in vivo* and *in vitro* is the presence or lack of the gastric mucosa and, possibly, also the submucous plexus. The possibility has to be considered that, in addition to its direct effect on gastric smooth muscle, one of the tachykinins tested has an indirect action via some submucous or mucosal structure, thus reducing the effect of SP or potentiating the effect of NKA.

Finally, since SP was recently shown to be able to stimulate visceral sensory receptors (Lew & Longhurst, 1986), it might, in the *in vivo* preparations, evoke a vagal reflex response, thus superimposing relaxation on the SP-induced contraction. It is not yet known, though, whether or not NKA shares this property with SP.

In conclusion, it has been shown that, in the rat stomach, NKA acts directly on circular smooth muscle and is more potent than SP, which appears to act partly via cholinergic interneurones. The role of tachykinins derived from intrinsic neurones and that of tachykinins from sensory neurones remain to be established.

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