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TACHYKININS AND BOMBESIN EXCITE NON-PYRAMIDAL NEURONES IN RAT HIPPOCAMPUS

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SUMMARY

1. The effects of substance P, eledoisin and physalaemin – which are structurally similar and all belong to the tachykinin family – and of bombesin, a gastrin-releasing peptide, on non-pyramidal neurones were studied using unitary extracellular recordings from rat hippocampal slices. The peptides were added to the perifusion solution, or locally applied by pressure ejection from a micropipette, at concentrations ranging from 10^{-8} to 10^{-6} M.

2. 104 out of 115 non-pyramidal neurones responded to tachykinins, and 26 out of 27 responded to bombesin, by a reversible, concentration-dependent increase in firing.

3. The responsive neurones retained their sensitivity to the tachykinins and to bombesin under the condition of synaptic blockade.

4. A synthetic peptide known to antagonize the effects of oxytocin on hippocampal non-pyramidal neurones did not affect the excitations induced by the tachykinins or bombesin. The action of the tachykinins was not blocked by the muscarinic antagonist, atropine.

5. These results indicate that hippocampal non-pyramidal neurones – which were previously shown to possess oxytocin receptors and μ-type opiate receptors – bear receptors for peptides of the tachykinin and of the gastrin-releasing families.

6. The hippocampal effects of tachykinins and of bombesin, however, were not blocked by synthetic structural analogues of substance P, known to antagonize the action of these peptides on some non-nervous tissues. The possibility must be considered that brain receptors for tachykinins and for gastrin-releasing peptides may be distinct from the peripheral receptors for these peptides.

INTRODUCTION

Substance P, eledoisin and physalaemin are undecapeptides of mammalian, molluscan and amphibian origin, respectively, and have a common C-terminus amino acid sequence. They are members of the tachykinin family, as is kassinin, a dodecapeptide isolated from amphibians (Harmar, 1984). Recently, two novel tachykinins have been isolated from mammalian tissues: substance K (or neurokinin A; Maggio, Sandberg, Bradley, Iversen, Santikarn, Williams, Hunter & Hanley,

1983; Kimura, Okada, Sugita, Kanazawa & Munekata, 1983) and neuromedin K (or neurokinin B; Kimura *et al.* 1983; Kangawa, Minamino, Fukuda & Matsuo, 1983). Using recombinant DNA techniques, two distinct tachykinin precursors from mammalian brain have been characterized (Nawa, Hirose, Takashima, Inayama & Nakanishi, 1983). One precursor contains the sequence of substance P, while the other bears the sequence both of substance P and of neurokinin A. In mammals, substance P has been detected in the gut, in the bronchia and in the eye; it is also present in sympathetic ganglia as well as in many parts of the central nervous system (for a recent review, see Pernow, 1983).

The tetradecapeptide bombesin (Anastasi, Erspamer & Bucci, 1971) and the undecapeptide ranatensin (Nakajima, Tanimura & Pisano, 1970) are both of amphibian origin and belong to the gastrin-releasing peptide family. Bombesin-like peptides have been found in the mammalian gastro-intestinal tract and brain (Minamino, Kangawa & Matsuo, 1983).

Peptides of both families have been found in the rat hippocampus. This brain area, and in particular its ventral subdivision, contains scattered substance P immunoreactive fibres (Roberts, Woodhams, Polak & Crow, 1984). Binding sites for radiolabelled substance P or physalaemin have been detected in the hippocampus (Rothman, Herkenham, Pert, Liang & Cascieri, 1984*b*; Mantyh, Hunt & Maggio, 1984*a*; Shults, Quirion, Chronwall, Chase & O'Donohue, 1984; Wolf, Moody, Quirion & O'Donohue, 1985). Moreover, the distribution of substance P binding sites present in this as well as in other brain regions has been shown to correlate with the amount of substance P-induced hydrolysis of inositol phospholipids in the same areas (Mantyh, Pinnock, Downes, Goedert & Hunt, 1984*c*). The rat hippocampus also contains numerous bombesin binding sites (Zarbin, Kuhar, O'Donohue, Wolf & Moody, 1985) and, in its ventral part, densely staining ranatensin immunoreactive fibres (Chronwall, Pisano, Bishop, Moody & O'Donohue, 1985) probably originating from the dorsal tegmental pons (Chronwall, Skirboll & O'Donohue, 1985).

All these data suggest that tachykinins and gastrin-releasing peptides may act as neurotransmitters in the hippocampus. However, according to a study of Dodd & Kelly (1981), rat hippocampal pyramidal neurones are unresponsive to substance P. We have examined the actions of tachykinins and of bombesin in the hippocampus by assessing their effects on non-pyramidal neurones. Amongst these cells are inhibitory interneurones, known to respond to neurohypophysial peptides by increased firing (Mühlethaler, Sawyer, Manning & Dreifuss, 1983; Mühlethaler, Charpak & Dreifuss, 1984) and to opioid peptides by a decrease in firing rate (Raggenbass, Wuarin, Gähwiler & Dreifuss, 1985*b*). We report in this article that the same non-pyramidal neurones are also excited by tachykinins and by bombesin. Preliminary communications of some of these results have been presented (Raggenbass, Wuarin & Dreifuss, 1985*a*; Dreifuss, Raggenbass & Wuarin, 1985).

METHODS

Hippocampal slices

Slices from the ventral hippocampus were obtained from male Sivz rats, weighing 200–250 g (Sivz is a Sprague–Dawley-derived strain). The animals were decapitated, their brain was rapidly removed, one hippocampus was dissected and 350–400 μm thick transverse slices were cut with a

Sorvall tissue chopper. The slices were transfered to a thermoregulated (35–36 °C) recording chamber and laid down on a nylon grid at the interface between a humidified oxygenated atmosphere and a perifusion medium (NaCl, 130 mM; KCl, 5 mM; $NaHCO_3$, 20 mM; $MgSO_4$, 2 mM; KH_2PO_4, 1·2 mM; glucose, 10 mM and $CaCl_2$, 1 mM), bubbled with 95% O_2, 5% CO_2. Under these conditions, the pH in the recording chamber was within the range 7·35–7·45. The solution flowed at a rate of 2 ml/min, and could be entirely substituted in about 2·5 min. Recordings were started after the preparation was allowed to recover for at least 1 h.

Electrophysiological recordings

Extracellular recordings were obtained from stratum pyramidale in the CA1/subicular region, using glass micropipettes filled with 4 M-NaCl and having a tip resistance of 4–20 MΩ. Signals were filtered (band width: 0·1–3·0 kHz) and displayed on an oscilloscope. Rate-meter records of single-cell firing were plotted on paper. Stimuli were applied by using either twisted bipolar electrodes (made of nichrome wires, 100 μm in diameter, and isolated except at the tip), or concentric bipolar electrodes (MCE-100, Rhodes Medical Instruments). Stimulation electrodes were positioned in stratum radiatum, and orthodromic action potentials were elicited in Schaffer's collaterals with constant current pulses (10–750 μA, 0·1 ms), delivered at frequencies ranging from 0·03 to 1 Hz. To monitor evoked field potentials, recorded signals were displayed on an oscilloscope under d.c. conditions.

Chemicals

Substance P, eledoisin, physalaemin and bombesin were purchased from Bachem, Bubendorf, Switzerland and from Serva, Heidelberg, F.R.G. Oxytocin, [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-SP and [D-Arg[1],D-Trp[7,9],Leu[11]]-SP (Spantide) were from Bachem. d[Tyr(Me)[2],Val[4],D-Arg[8]]-vasopressin was a gift from Dr M. M. Manning (Department of Biochemistry, Medical College of Ohio, Toledo, U.S.A.). Atropine sulphate monohydrate was purchased from Fluka, Buchs, Switzerland.

Usually the drugs were tested after dissolving them in the perifusion medium. In some instances, they were pressure-ejected from a glass pipette (20–30 μm in tip diameter), placed near the recording electrode. Ejection was achieved by applying pressure pulses (10–50 kPa, 100–250 ms) at a rate of 1 Hz, for the duration of time indicated. When the drugs were pressure ejected, the concentration indicated is the concentration in the solution contained in the pipette.

RESULTS

The action of peptides of the tachykinin family on non-pyramidal neurones was studied by using unitary extracellular recordings from rat hippocampal slices. Non-pyramidal neurones were distinguished from pyramidal neurones by using previously defined criteria (Mühlethaler *et al.* 1984). Non-pyramidal neurones displayed small and short action potentials. Nearly all were spontaneously active, and discharged at a mean rate of about 15 spikes/s. Usually, they responded to stimulation of stratum radiatum by a train of spikes, whose duration outlasted that of the pyramidal cell population spike. In contrast, pyramidal neurones were silent, and responded to the same stimulation by a single action potential, at a latency comparable to that of the population spike.

In total 115 non-pyramidal neurones were recorded from 83 slices. 104 neurones responded by a reversible increase in firing to substance P, eledoisin or physalaemin (Fig. 1); 4 responded by a decrease in firing, and 7 did not respond at all. The excitation was concentration dependent. Substance P, eledoisin and physalaemin were approximately equipotent, and therefore these data were pooled (Table 1). Within the range of the concentrations tested, a plateau was not obtained for the effects of the tachykinins, the increase in firing at 10^{-6} M being significantly greater

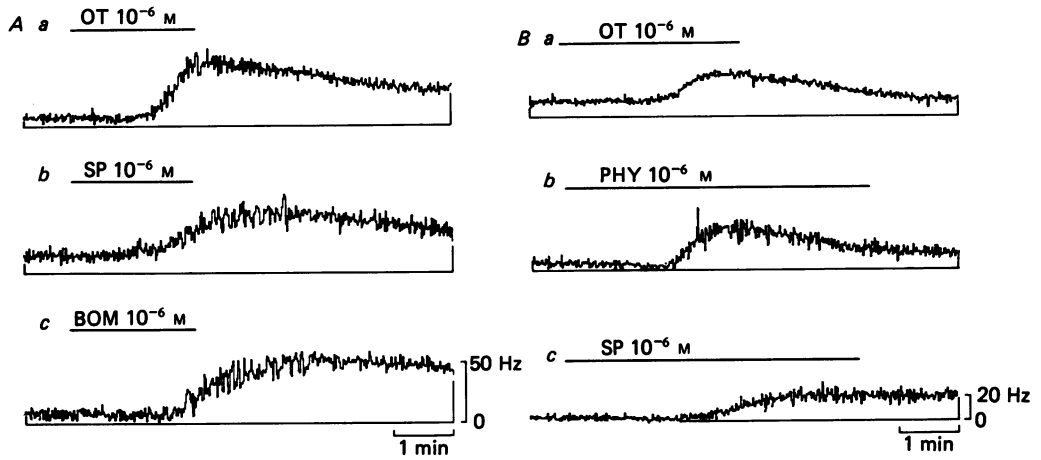

Fig. 1. Effects of oxytocin (OT), substance P (SP), bombesin (BOM) and physalaemin (PHY) on two hippocampal non-pyramidal neurones, *A* and *B*. Each peptide was added to the perifusion solution at 10^{-6} M and for the time shown by the continuous line above each trace. Records *Aa–c* are consecutive but not contiguous, as are *Ba–c*. In all cases, the peptide-induced increase in firing rate was fully reversible. Note that for neurone *B* the resting firing rate decreased progressively with time.

TABLE 1. Effects of tachykinins (substance P, eledoisin and physalaemin), of bombesin and of oxytocin on the firing of hippocampal non-pyramidal neurones

	Concentration (M)*					
Peptide	10^{-9}	10^{-8}	5×10^{-8}	10^{-7}	5×10^{-7}	10^{-6}
Tachykinins						
Increase in firing rate (spikes/s)†	—‡	0	16	21	34§	57§
S.E. of mean (spike/s)	—	0	5	3	4	6
Sample size	—	2	4	13	15	20
Bombesin						
Increase in firing rate (spikes/s)†	—‡	8	—‡	29	46	47
S.E. of mean (spike/s)	—	7	—	8	17	9
Sample size	—	4	—	7	3	6
Oxytocin						
Increase in firing rate (spikes/s)†	12	34	—‡	48	—‡	49
S.E. of mean (spike/s)	5	7	—	7	—	5
Sample size	4	4	—	9	—	11

* The peptide was dissolved in the perifusion solution at the concentration indicated.

† The effect is the mean increase in firing above the resting rate of 13 ± 2 spikes/s (mean ± S.E. of mean). The data were obtained from thirty neurones in twenty-six slices. The potencies of substance P (tested on seven neurones), eledoisin (eight neurones) and physalaemin (eleven neurones) were indistinguishable; therefore their respective data were pooled to give one single concentration–response relation.

‡ Not tested on these neurones.

§ These two values are significantly different ($P < 0.005$, Student's t test).

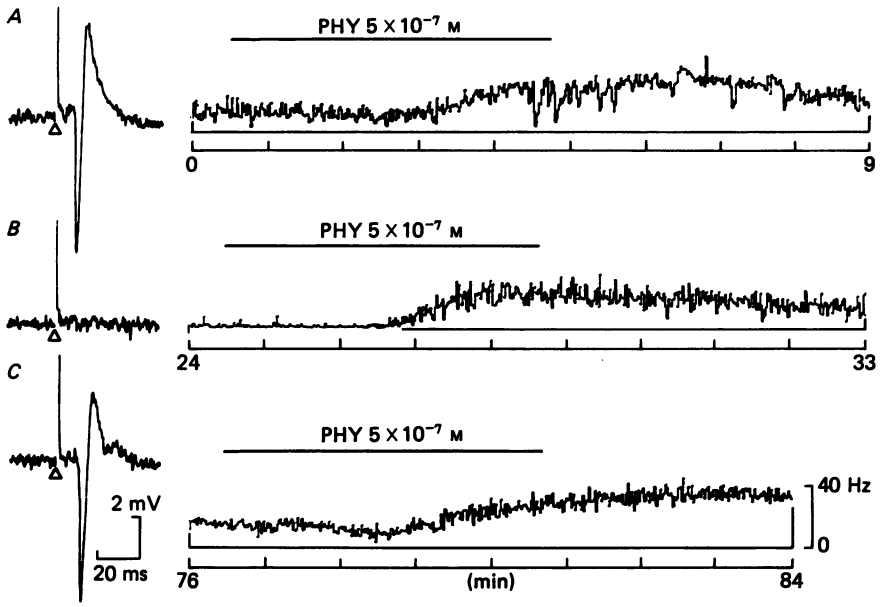

Fig. 2. Effects of physalaemin (PHY) on the firing of a non-pyramidal hippocampal neurone in normal medium (right panels, *A* and *C*), and in a modified medium containing 0·2 mM-$CaCl_2$ and 6 mM-$MgSO_4$ (right panel, *B*). The preparation was perifused with the modified medium during 20 min. To monitor the efficacy of synaptic coupling, orthodromically evoked field potentials were recorded at 14 min (left panel, *A*), at 24 min (left panel, *B*) and at 76 min (left panel, *C*). Each stimulus artifact is marked by an open triangle. Note that in *B* the evoked potential disappeared, the spontaneous activity of the neurone was markedly reduced, but physalaemin still excited the cell.

than that obtained at 5×10^{-7} M (Table 1). Concentrations of peptides higher than 10^{-6} M were not used, since under such conditions non-specific effects might predominate. Since the concentration–response curve for the tachykinins did not reach a plateau, we could not define a half-maximal concentration of their electrophysiological effects. However, in view of the results shown in Table 1, we conclude that it must be equal to or greater than 10^{-7} M.

Tachykinin-sensitive non-pyramidal neurones were also tested for oxytocin, the more potent of the two neurohypophysial peptides on these cells (Mühlethaler *et al.* 1983). All fifty-five neurones tested responded to oxytocin with an increase in firing (Fig. 1). Comparison of the concentration–response relations for the tachykinins and for oxytocin shows that the tachykinins were 50–100 times less potent than oxytoxcin (Table 1).

To determine whether the excitatory action of tachykinins was direct or indirect, the responsiveness of non-pyramidal neurones to tachykinins was tested under the condition of synaptic uncoupling. This was achieved by replacing the perifusion solution with a modified medium containing 0·2 mM-$CaCl_2$ and 6 mM-$MgSO_4$ (instead of 1 mM-$CaCl_2$ and 2 mM-$MgSO_4$). Under this condition two reversible changes took place: (i) the synaptically elicited field potential and the population spike disappeared; (ii) non-pyramidal neurones either became silent or acquired a slower mean firing rate

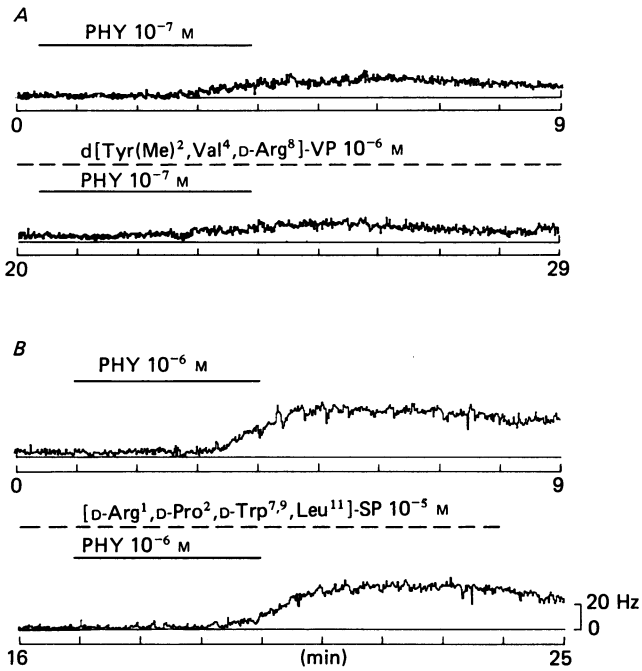

Fig. 3. Effects of physalaemin (PHY) and of two synthetic structural analogues, d[Tyr(Me)^2,Val^4,D-Arg^8]-vasopressin (VP) and [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP, on the firing of two hippocampal non-pyramidal neurones, *A* and *B*. Physalaemin was added to the perifusion solution at the concentrations and for the periods indicated. In *A*, the analogue was present at 10^{-6} M for 15 min, starting from the 15th min. On this neurone, this same compound at 10^{-6} M completely and reversibly antagonized the effect of oxytocin at 10^{-7} M (not shown). In *B*, the analogue was present at 10^{-5} M for 10 min, starting from the 14th minute.

with a bursting firing pattern. In all six experiments, addition of tachykinins to the perifusion medium at 10^{-7}–10^{-6} M either reactivated the silenced neurones or increased the firing of those which had remained spontaneously active (Fig. 2).

Effects of antagonists on the response to tachykinins

The excitatory effects of oxytocin at 10^{-7} M on non-pyramidal neurones were completely and reversibly antagonized by the synthetic structural analogue d[Tyr(Me)^2,Val^4,D-Arg^8]-vasopressin, added to the perifusion solution at 10^{-6} M. In contrast, in five neurones tested, this analogue at the same concentration did not affect the increase in firing induced either by physalaemin (Fig. 3*A*) or by substance P at 10^{-7} M.

To assess whether the effect of tachykinins might be due to an indirect action expressed through cholinergic receptors, we applied the muscarinic antagonist, atropine. At concentrations ranging from 10^{-7} to 10^{-5} M, this compound, in seven cells, neither affected the resting firing rate nor blocked the increase in firing brought about by tachykinins at 10^{-7}–10^{-6} M.

The effects of the structural analogues [D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]]-SP and [D-Arg[1],D-Trp[7,9],Leu[11]]-SP (Spantide) on the tachykinin-induced excitation of non-pyramidal hippocampal neurones were tested. These compounds were added to the perifusion solution at concentrations ranging from 10^{-6} to 2×10^{-5} M. When both a structural analogue and a tachykinin were present, the ratio between their respective concentrations ranged from 10 to 1000. In twelve out of twelve non-pyramidal neurones tested, the structural analogues neither affected the spontaneous firing of the neurones, nor did they antagonize the increase in firing induced by the tachykinins (Fig. 3*B*).

Effects of bombesin

Bombesin increased the rate of firing of twenty-six out of twenty-seven non-pyramidal neurones (Fig. 1). The concentration used ranged from 10^{-8} to 10^{-6} M. Usually, the bombesin-induced excitation was fully reversible. However, in a few instances (especially when high concentrations of peptide were used) recovery of the resting firing rate was very slow (10 min or more) and sometimes incomplete. Bombesin excited non-pyramidal neurones in a concentration-dependent manner (Table 1). Half-maximal effects were attained around 10^{-7} M.

All fourteen bombesin-sensitive neurones that were tested also responded to oxytocin (at 10^{-8}–10^{-6} M), and nine out of nine also responded to tachykinins (at 10^{-7}–10^{-6} M). Similar to the tachykinins, bombesin was less potent than oxytocin (Table 1). The effects of bombesin (at 10^{-7} M) were not altered by the structural analogue d[Tyr(Me)[2],Val[4],D-Arg[8]]-vasopressin at 10^{-6} M (tested on three neurones). Neither were the bombesin effects (at 10^{-7}–10^{-6} M) antagonized by the two previously mentioned substance P structural analogues, added to the perifusion medium at 10^{-5}–2×10^{-5} M (tested on four neurones). Finally, neurones which responded to bombesin were still excited by this peptide under the condition of synaptic uncoupling (tested in three experiments).

DISCUSSION

We have shown that a population of hippocampal non-pyramidal neurones respond to tachykinins (substance P, eledoisin and physalaemin) and to bombesin in a direct, concentration-dependent and specific way. We conclude that these neurones probably possess receptors for tachykinins and for peptides of the gastrin-releasing family. The same neuronal population has been previously shown to bear oxytocin receptors (Mühlethaler *et al.* 1984) and μ-type opiate receptors (Raggenbass *et al.* 1985*b*).

Apparent dissociation constants (K_D) ranging from 3×10^{-10} to 7×10^{-9} M were obtained for radiolabelled substance P or physalaemin bound to brain slices (Rothman *et al.* 1984*b*; Mantyh *et al.* 1984*a*; Schults *et al.* 1984; Wolf *et al.* 1985; Mohini, Bahouth, Brundish & Musacchio, 1985), whereas radiolabelled bombesin binding had an apparent K_D of 4–6×10^{-9} M (Zarbin *et al.* 1985). The relatively high apparent K_D values for the electrophysiological effects may be due to several factors. (i) Peptide inactivation may have occurred in the recording chamber, either by enzymatic degradation or by conversion to less potent oxydized forms (Mantyh *et al.* 1984*a*). (ii) The peptides used in the present study may not be amongst the most

powerful agonists of hippocampal tachykinin or bombesin receptors. Related peptides exist (see Introduction), some of which may possess higher potencies. In this respect, however, and with regard to the tachykinins, preliminary experiments performed in this laboratory indicate that neither kassinin nor various C-terminal fragments of substance P have significantly higher potency than substance P, eledoisin or physalaemin.

Tachykinin receptor subtypes

Recently, a classification of substance P receptors into subtypes has been proposed (Iversen, Hanley, Sandberg, Lee, Pinnock & Watson, 1982). It is based on the relative potencies shown by the different tachykinins in inducing smooth muscle contraction. Thus, receptors of the P subtype were defined as those on which substance P, eledoisin and physalaemin were equipotent, whereas receptors of the E subtype were those on which eledoisin was clearly more potent than the two other tachykinins. In addition, autoradiographic data suggest the existence of several distinct tachykinin binding sites in the rat brain. One class of sites binds efficiently substance P, whereas other classes show low affinities for substance P, but bind preferentially eledoisin (Rothman, Danks, Herkenham, Cascieri, Chicchi, Liang & Pert, 1984*a*) or kassinin (Mantyh, Maggio & Hunt, 1984*b*). Both the high-affinity and the low-affinity binding sites for substance P have been found in the hippocampus (Mantyh *et al.* 1984*b*) and it has been proposed that the two classes of tachykinin binding sites correspond to the functionally defined P and E receptor subtypes (Rothman *et al.* 1984*a*). In terms of this dichotomy, the excitation of non-pyramidal neurones described in the present article is more likely to result from an interaction with receptors of the P rather than the E subtype. However, such a conclusion is only tentative. Indeed, the sub-division of substance P receptors into two functional subclasses is based solely on agonist potency profiles and has not yet been confirmed by the discovery of selective antagonists (Watson, 1984). In addition, the recent isolation of the novel mammalian tachykinins, substance K (or neurokinin A) and neuromedin K (or neurokinin B), raises the possibility that additional subtypes of tachykinin receptors may exist, distinct from the P and the E subtypes (Buck, Burcher, Shults, Lovenberg & O'Donohue, 1984).

Tachykinins and bombesin antagonists

The hippocampal actions of tachykinins and bombesin described in the Results section were not suppressed by structural analogues of substance P known to antagonize some effects of the tachykinins on smooth muscle (Folkers, Håkanson, Hörig, Xu & Leander, 1984) and of bombesin in the pancreas (Jensen, Jones, Folkers & Gardner, 1984). This suggests that the hippocampal receptors for tachykinins and gastrin-releasing peptides may be distinct from the peripheral receptors. However, although the structural analogues of substance P we used are amongst the most potent tachykinin and bombesin antagonists available, their apparent affinity for peripheral receptors is still 1000- to 10000-fold lower than that of the respective agonists. Therefore, the possibility remains that we did not detect antagonistic effects of the substance P structural analogues since, in our conditions, the highest concentration at which we could test them was 2×10^{-5} M.

In studying possible antagonistic effects of substance P analogues in the nervous system, similar negative results have also been reported by other groups. The analogue [D-Pro^2,D-Trp^{7,9}]-SP, which is able to antagonize substance P-induced smooth muscle contraction (Björkroth, Rosell, Xu & Folkers, 1982), was ineffective when tested for substance P antagonism both in the caudal trigeminal nucleus *in situ* and in the isolated spinal cord *in vitro* (Salt, De Vries, Rodriguez, Cahusac, Morris & Hill, 1982). Using brain-stem slices, Cheeseman, Pinnock & Henderson (1983) showed that this same synthetic analogue did not counteract the excitation of locus ceruleus neurones brought about by substance P, although in a previous study Engberg, Svensson, Rosell & Folkers (1981) had reported that this analogue could block the substance P-induced excitation of these neurones *in situ*. Neither [D-Pro^4,D-Trp^{7,9}]-SP nor [D-Pro^4,D-Trp^{7,9,10}]-SP (Caranikas, Mizrani, D'Orléans-Juste & Regoli, 1982) antagonized the excitatory effects of iontophoretically applied substance P on neurones of the nucleus tractus solitarius of the cat (Morin-Surun, Jordan, Champagnat, Spyer & Denavit-Saubie, 1984). In addition, [D-Arg^1,D-Trp^{7,9},Leu^{11}]-SP as well as two novel structural analogues, [Arg^5,D-Trp^{7,9},Nle^{11}]-SP(5–11) and [Arg^5,Ala^6,D-Trp^{7,9},Nle^{11}]-SP(5–11), were devoid of any substance P antagonistic activity in the spinal cord from neonatal rats and superior cervical ganglion *in vitro* (Brown, Calthrop, Hawcock & Jordan, 1985). These results, as well as our own, indicate that more potent, selective antagonists of tachykinins and of gastrin-releasing peptides are needed in order to substantiate the existence of, and to further characterize, the receptors mediating the excitatory actions of these peptides in the nervous system. At variance with the results cited above, however, both [D-Pro^2,D-Trp^{7,9}]-SP and [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP markedly reduced the depolarization induced by brief pulses of substance P applied either to motoneurones in the rat neonatal spinal cord *in vitro* (Yanagisawa, Otsuka, Konishi, Akagi, Folkers & Rosell, 1982; Matsuto, Yanagisawa, Otsuka, Kanazawa & Munekata, 1984; Akagi, Konishi, Otsuka & Yanagisawa, 1985) or to neurones of the guinea-pig inferior mesenteric ganglion *in vitro* (Konishi & Otsuka, 1985). The reason for this discrepancy is unclear, although it has been suggested (Brown *et al.* 1985) that it may be related to the mode of application of the agonists (cf. Matsuto *et al.* 1984).

Concluding remarks

The results presented in this article, combined with previous electrophysiological, autoradiographical and immunocytochemical data, suggest that tachykinins, gastrin-releasing peptides, neurohypophysial hormones as well as opioid peptides may serve as neurotransmitters in the rat hippocampus, and have non-pyramidal neurones (or, at any rate, a proportion of them) as their common target.

Studies carried out using intracellular recording techniques have shown that substance P can excite bull-frog sympathetic neurones (Adams, Brown & Jones, 1983; Akasu, Nishimura & Koketsu, 1983), rat (Murase & Randić, 1984) and mouse spinal cord neurones (Nowak & MacDonald, 1982), rat globus pallidus neurones (Stanfield, Nakajima & Yamaguchi, 1985), as well as guinea-pig hypothalamic neurones (Ogata & Abe, 1982) by depolarizing their plasma membrane. It was found that the substance P-induced membrane depolarization was associated with a decrease of membrane voltage-dependent potassium conductance(s). In order to

assess whether the same mechanism causes the tachykinin-induced excitation of hippocampal non-pyramidal neurones, intracellular recordings will be needed.

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