The selective NK_3 receptor agonist senktide excites a subpopulation of dopamine-sensitive neurones in the rat substantia nigra pars compacta *in vitro*

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Intra- and extracellular recordings were made from substantia nigra zona compacta neurones from an *in vitro* rat brain slice preparation. A subpopulation of dopamine-sensitive neurones were encountered which were potently excited by bath application of the NK_3 receptor agonist, senktide. On these senktide-sensitive neurones, NK_1 and NK_2 receptor agonists were inactive. The excitatory action of senktide supports a role for tachykinins as putative neurotransmitters in the basal ganglia.

Keywords: Basal ganglia; substantia nigra; dopamine; tachykinin; senktide; neurokinin

Introduction There is some evidence for the existence of a striatonigral pathway which utilizes substance P (SP) as a neurotransmitter (Dray, 1980). The highest concentrations of SP-like immunoreactivity (SPLI) occur in the substantia nigra (Ljungdahl *et al.*, 1978) and it has been shown that injection of SP into the substantia nigra results in increases in dopamine cell activity as measured by changes in dopamine release and turnover (Cheramy *et al.*, 1978). In this study we have made use of tachykinin receptor-selective agonists to study the action of SP on substantia nigra compacta (SNC) neurones which respond to dopamine. These have allowed us partially to characterize the receptor type involved in the tachykinin response in the substantia nigra, pars compacta.

Methods Experiments were performed on brain slices (300 μm thick) from 150 g male Wistar rats and extracellular recordings were made by conventional techniques (Keegan *et al.*, 1990). Drugs were applied by bath perfusion and removal was achieved simply by returning to the control drug-free solution. There was a 'dead time' of 1 min due to the perfusion process. Extracellular electrodes were filled with aCSF and

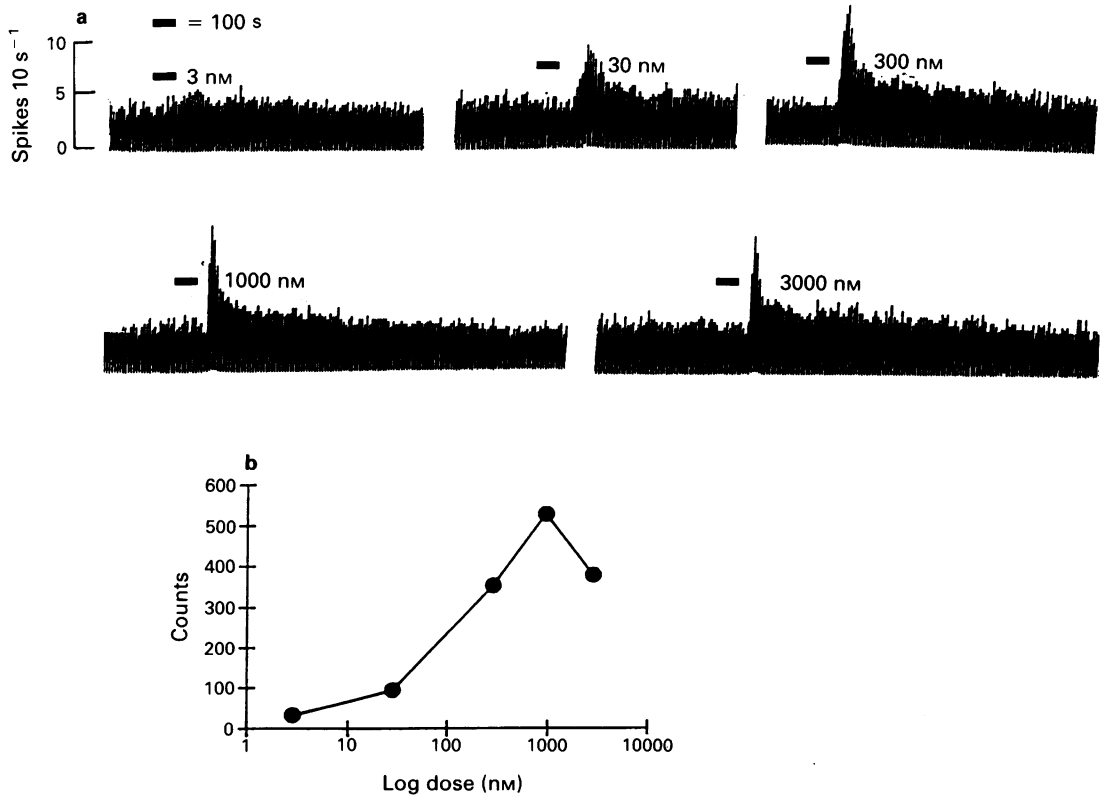

Figure 1 Ratemeter histogram from a substantia nigra neurone showing that the excitatory actions of senktide are concentration-dependent and long lasting. (a) The ratemeter recordings were taken from the same neurone, the responses plotted on the graph (b) were obtained by using the total number of action potentials occurring over a 20 min period and subtracting the number of action potentials occurring over a 20 min control period.

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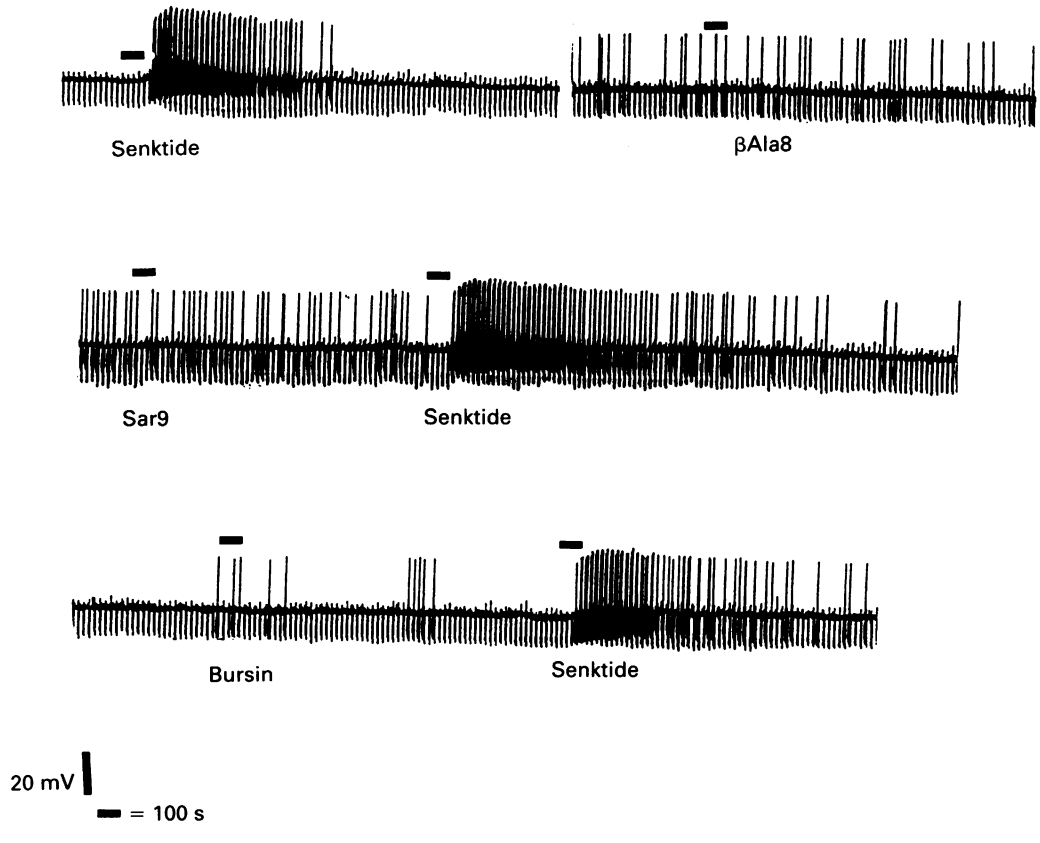

Figure 2 Intracellular recording from a substantia nigra neurone showing that the excitatory response to senktide (500 nM) was caused by a depolarization of the membrane potential without a large change in membrane conductance and the lack of effect of the NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]SP(1-11) (1 μM) and the NK_2 receptor agonist [βAla^8]NKA(4–10) (1 μM) on the same neurone. The downward deflections of membrane potential were caused by the injection of constant current (0.2 Hz, 200 ms, 0.5 nA) through the recording electrode. The resting potential of the cell was −51 mV.

had resistances of 5–14 MΩ. For intracellular recordings, electrodes were filled with 1 M potassium acetate and had d.c. resistances of 70–110 MΩ. Neurones were considered to be dopaminergic if they had a characteristic waveform, slow firing rate (~5 Hz) and inhibitory response to dopamine (Pinnock, 1985). β-Ala^8 was obtained from Novabiochem. All other drugs were obtained from Peninsula laboratories.

Results The selective NK_3 receptor agonist senktide (succinyl-[Asp^6, $MePhe^8$]SP(6-11)) excited 24 of 31 dopaminergic neurones in the substantia nigra pars compacta in a concentration-dependent manner. The effective concentration-range was between 3–3000 nM. The mean EC_{50} for senktide was 41.2 ± 9 nM (n = 5). Senktide responses were prolonged and required 10–30 min before recovery (Figure 1). At concentrations greater than 1 μM, there was a marked degree of desensitization. The selective NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]SP(1-11) and the selective NK_2 receptor agonist, [βAla^8]NKA(4-10) were inactive on senktide-sensitive neurones (n = 6; Figure 2) within the concentration-range used (500–3000 nM). In contrast to the potent actions of senktide, SP was only weakly active on senktide-sensitive neurones with responses observed only when concentrations greater than 1 μM were applied (n = 3). The peptide, bursin, was inactive on all three senktide-sensitive neurones on which it was tested. Most senktide-sensitive neurones were encountered in coronal brain sections of the SNC at the level of the interpeduncular nucleus (18 of 24) whilst none were encountered as rostral as the level of the medial mamilliary nucleus.

Discussion Autoradiographic studies of the distribution of tachykinin receptors reveal a surprisingly poor relationship between the distribution of tachykinin receptors and SPLI although a good correlation between [^{3}H]-SP binding sites and inositol phospholipid hydrolysis has been reported (Mantyh *et al.*, 1984). Thus whilst the substantia nigra contains the highest levels of SPLI in the brain (Ljungdhal *et al.*, 1978) there is a sparse distribution of SP binding sites. In agreement with this observation, previous electrophysiological studies *in vivo* have demonstrated a weak excitatory action of SP on identified dopamine neurones of the SNC (Pinnock and Dray 1982). Furthermore, preliminary experiments *in vitro* suggest a differential sensitivity between the SNC and SNR neurones to substance P (Pinnock, 1983). One explanation for the relatively low potency of SP on SNC neurones may be that neurokinin B (NKB) acts as the endogenous ligand for the tachykinin receptor in the substantia nigra. Indeed this tachykinin appears to be the preferred agonist for the NK_3 receptor. Furthermore, the presence of NKB in the substantia nigra has been demonstrated by radioimmunochemical techniques (Kanazawa *et al.*, 1984). In conclusion this study demonstrates that a subpopulation of dopamine-sensitive neurones in the substantia nigra are sensitive to the selective NK_3 receptor agonist, senktide, but the lack of NK_3-selective antagonists prevents definitive characterization of the tachykinin receptor involved. Recent autoradiographic studies with [^{3}H]-senktide have also revealed NK_3 binding sites in the SNC (Dam *et al.*, 1990). The absence of responses to the NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]SP(1-11) and the NK_2 receptor agonist [βAla^8]NKA(4-10) suggests that NK_1 and NK_2 receptors are not present on dopamine neurones in the SNC. These results support a role for tachykinins as putative neurotransmitters in the basal ganglia.

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