ELSEVIER

European Journal of Pharmacology 273 (1995) 113–119

ejp

Pharmacology of tachykinin receptors on neurones in the ventral tegmental area of rat brain slices

Guy R. Seabrook *, Barbara J. Bowery, Raymond G. Hill

Department of Pharmacology, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK

Received 11 August 1994; revised 21 October 1994; accepted 1 November 1994

Abstract

The pharmacology of tachykinin receptors within the ventral tegmental area of rat brain slices was studied using in vitro electrophysiological techniques. The selective tachykinin NK_3 receptor agonist senktide (100 nM) increased the action potential firing rate from 1.9 to 3.9 Hz in 70% of spontaneously active cells tested ($n = 27$). Senktide was the most potent agonist tested with an EC_{50} of 4 nM. In contrast the NK_1 receptor agonists substance P-O-methyl ester (100–300 nM) or GR 73632 (1 μM) were inactive at the concentrations tested. Responses to neurokinin B ($EC_{50} = 32$ nM) were not blocked by the tachykinin NK_1 receptor antagonist CP 99,994 (1 μM) nor by the tachykinin NK_2 receptor antagonist SR 48968 (300 nM). Similarly responses to the tachykinin NK_2 receptor agonist β-[Ala^8]neurokinin A-(4–10) ($EC_{50} = 427$ nM) were not antagonised by the tachykinin NK_2 receptor antagonist SR 48968 (300 nM) and thus were likely to be due to the activation of tachykinin NK_3 receptors. These data demonstrate that NK_3, and not NK_1 or NK_2 receptors, mediate the principal excitatory effects of exogenously applied tachykinin receptor agonists on dopamine neurones within the rat ventral tegmental area.

Keywords: Dopamine; Electrophysiology; Neurokinin; Senktide; Substance P; Tachykinin; Ventral tegmental area

1. Introduction

Mammalian tachykinins act upon several types of pharmacologically defined tachykinin receptors, three of which have recently been cloned. These cloned receptors are designated NK_1, NK_2, and NK_3, and they are preferentially although not exclusively activated by the endogenous peptides substance P, neurokinin A, and neurokinin B, respectively (reviewed in Iversen et al., 1990). The localisation of these tachykinins and their target receptors in brain tissue have been extensively studied using monoclonal antibodies, radioligand binding, and more recently in-situ hybridisation techniques (Mantyh et al., 1989; Dam et al., 1990; Gerfen, 1991; Humpel and Saria, 1993). However only limited information is available about the functional significance of these receptors in brain tissue.

Substance P and neurokinin B increase the excitability of neurones in several areas of the central nervous system. These areas include the locus coeruleus (Guyenet and Aghajanian, 1977; Seabrook et al., 1993), medial habenula (Norris et al., 1993), and raphe nuclei (Pomeroy and Behbehani, 1980). Furthermore several studies have shown that tachykinins excite midbrain dopamine neurones in the substantia nigra pars compacta and ventral tegmental area (e.g. Davies and Dray, 1976; Pinnock and Dray, 1982). However the receptor subtype(s) that regulate the excitability of these neurones are not fully understood. Some studies have implicated predominantly NK_3 tachykinin receptors (Keegan et al., 1992), whereas others have suggested that NK_1 and NK_2 tachykinin receptors may also influence the excitability of midbrain dopamine neurones (Overton et al., 1992). Some of these differences may simply be a consequence of the different brain regions studied (substantia nigra versus ventral

* Corresponding author. Tel. (0279) 440479, fax (0279) 440390.

Elsevier Science B.V.
SSDI 0014-2999(94)00681-4

tegmental area respectively), or indeed to receptor population differences between the dopamine and γ-aminobutyric acid containing neurones which are present in both the substantia nigra pars compacta (Lacey et al., 1989) and ventral tegmental area (Johnson and North, 1992).

Consistent with a heterogeneity of tachykinin receptor subtypes between neurones, Pinnock and Dray (1982) showed that presumed dopamine- containing cells of the substantia nigra pars compacta were less sensitive to electrophoretically applied substance P than the γ-aminobutyric acid- containing cells of the pars reticulata. Neurones within both of these areas are also excited by electrophoretically applied neurokinin A, a tachykinin NK_2 receptor agonist (Innis et al., 1985). Overton et al. (1992), have also reported that the majority of neurones within the pars compacta were excited by focal application of GR64349, a NK_2 tachykinin receptor agonist, whereas within ventral tegmental area the majority were excited by NK_1, NK_2 and NK_3 tachykinin receptor agonists. However, a major complication with these studies is that agonists were applied by either electrophoresis or pressure ejection and thus the concentration of drug at the receptor was unknown. The majority of tachykinin receptor agonists can interact with more than one receptor subtype at micromolar concentrations, and hence it is difficult to assess their relative roles. Indeed, using a submerged brain slice preparation Keegan et al. (1992), showed that the selective tachykinin NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]substance P-(1–11) was inactive on most substantia nigra neurones whereas the selective tachykinin NK_3 receptor agonist senktide activated > 60% cells examined.

In contrast to the well described functional role of tachykinins in the substantia nigra the significance of NK_1 and NK_2 tachykinin receptors in the ventral tegmental area of rat brain needs to be elucidated. It is clear from both in vitro and in vivo studies that activation of NK_3 tachykinin receptors excites neurones in this nucleus and can induce locomotor, rearing, and sniffing behaviours (Stoessl et al., 1991; Overton et al., 1992). Despite this, at least one study has implicated NK_1 and not NK_2 or NK_3 tachykinin receptors as the principal tachykinin receptor subtype that mediates the excitatory influence on mesolimbic dopamine neurotransmission (Elliot et al., 1992), and it was suggested that antagonism of NK_1 tachykinin receptors on neurones within the ventral tegmental area may be useful in the treatment of psychosis. Consequently the present study was carried out in order to clarify the relative significance of tachykinin receptor subtypes on neurones in the rat ventral tegmental area using a submerged rat brain slice preparation to which selective agonists and antagonists were applied in known concentrations.

2. Materials and methods

2.1. Experimental preparations and recording techniques

Extracellular microelectrode recordings were made from 32 presumed dopamine-containing neurones in the ventral tegmental area of rat brain slices (300–350 μm thick). The techniques for preparation of, recording from, and application of substances to these neurones have previously been described (Pinnock, 1983; Lacey et al., 1988). In brief, coronal slices of rat ventral tegmental area (plate 25, Paxinos and Watson, 1944) were submerged in a tissue chamber (volume 0.4 ml) by a continuously superfused (at 1–2 ml min^{-1}) artificial cerebrospinal fluid kept at 36°C and gassed with 95% O_2 and 5% CO_2. Extracellular electrophysiological recordings were made with glass microelectrodes filled with 3 M NaCl (5–10 MΩ). Care was taken to ensure that recordings were made from within the ventral tegmental area, identified as medial to the terminal nucleus of the accessory optic tract and ventral to the medial lemniscus.

2.2. Analytical techniques

Drug effects were expressed as either the peak increase in cell firing (in Hertz) in response to a 1 min application of the agonist, or as the percent increase normalised to either the control firing rate or to a control application of senktide (100 nM) in each cell as indicated. Concentration-effect data were fitted to a logistic equation (e.g. Bowery et al., 1994) by least squares analysis of variance (Grafit; Erithacus Software). The agonist-induced increase in cell firing was determined using sequential application of increasing concentration of each agonist, allowing 30–40 min recovery to minimise tachyphylaxis. Data are expressed as the geometric mean ± S.E.M. All agonists were not tested on every cell, consequently the sample size for each ligand was less than the total number of cells studied ($n = 32$).

2.3. Drugs and solutions

The artificial cerebrospinal fluid contained (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10. Drugs were applied in the superfusion medium in known concentrations, reaching the recording chamber after a delay of 20–30 s. The following drugs were used: quinpirole hydrochloride (Research Biochemicals); senktide, [β-Ala^8]neurokinin A-(4–10), and neurokinin B (Cambridge Research Biochemicals). GR 73642 (δ-Ava[L-Pro^9,*N*-$MeLeu^{10}$]substance P-(7–11)), CP 99,994, (+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine (McLean et al., 1993), and SR 48968 (*S*)-*N*-methyl-*N*-

[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (Emonds-Alt et al., 1992) were synthesised at Merck Research Laboratories. CP 99,994 was used as the preferred tachykinin NK_1 receptor antagonist despite its relatively low affinity for rat NK_1 tachykinin receptors (IC_{50} = ca. 300 nM) compared to other species including guinea-pig and humans, because of its low affinity for calcium and sodium channels. Tachykinin receptor agonists were dissolved in distilled water (aliquoted stock solutions of 10 mM stored at −70°C), and antagonists were dissolved in dimethylsulphoxide (final bath concentration < 0.1% DMSO).

3. Results

Extracellular recordings were made from spontaneously active cells in the ventral tegmental area of rat brain slices. The slow time course of action potentials (2–5 ms) and firing rate of these cells (1–5 Hz) were consistent with those of principal, presumed dopamine-containing cells described previously (Johnson and North, 1992). Previous studies from our laboratory have shown that the spontaneous activity in most cells of this type are inhibited by application of the D_2 dopamine receptor agonist quinpirole (73/79 cells at 100 nM; Bowery et al., 1994).

Senktide, neurokinin B and [β-Ala^8]neurokinin A-(4–10) increased the firing rate in the majority of cells studied in a concentration-dependent manner. The half-maximally effective concentration of senktide, determined separately for each cell, was 4 nM (Fig. 1). The maximally effective concentration of senktide (100 nM) increased the basal firing rate from 1.9 ± 0.2 Hz to a peak of 3.9 ± 0.3 Hz (n = 19; averaged over a 10 s time interval). Responses to senktide were observed in 70% of cells examined (19/27 cells). In contrast no increase in firing rate of presumed dopamine neurones was detected in 17 cells tested with either substance P-O-methyl ester (0.1–1 μM) or GR 73632 (1 μM). Furthermore, 38% of cells did not respond to the application of either NK_1 or the NK_3 receptor agonist (5/13 cells), although the firing of these cells was inhibited by application of the D_2 dopamine receptor agonist quinpirole (100 nM, n = 5). In contrast to the responses of these presumed dopamine neurones, excitatory responses to substance P-O-methyl ester (100 nM) and not senktide (100 nM) were observed in putative non-dopaminergic cells, that were characterised by their high firing rates (> 3 Hz), fast time course of spontaneous action potentials (typically < 2 ms), and insensitivity to quinpirole (100 nM). These cells were found infrequently (< 5%) and thus the pharmacology of their tachykinin receptors was not studied in detail.

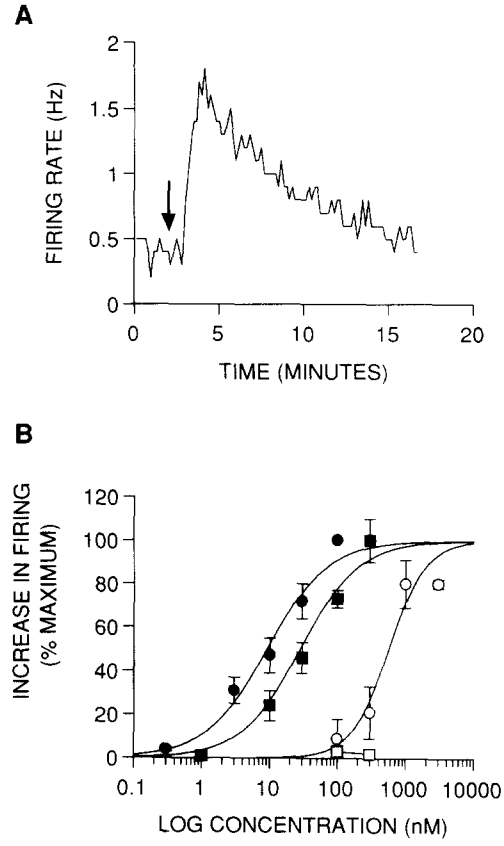

Fig. 1. Concentration-dependent increase in firing rate of spontaneously active cells within the ventral tegmental area of rat brain slices caused by senktide, neurokinin B, and [β-Ala^8]neurokinin A-(4–10). (A) Increase in action potential firing rate caused by neurokinin B (300 nM at arrow for 1 min) in an individual cell. Each point represents the rate averaged over a ten second period. (B) Pooled concentration-effect data from several cells expressed as the maximal increase in firing rate relative to 100 nM senktide. The half-maximally effective concentration of senktide (filled circles) was 4 nM, neurokinin B (filled squares) was 32 nM, and [β-Ala^8]neurokinin A-(4–10) (open circles) was 427 nM. Substance P-O-methyl ester was inactive on all presumed dopamine neurons that were tested (open squares). Data represent the means ± S.E.M from three to ten cells.

Like senktide, neurokinin B and [β-Ala^8]neurokinin A-(4–10) increased the firing rate of most cells studied. However, the half-maximally effective concentrations of these agonists were 8- and 107-fold higher respectively than that of senktide (Table 1). In addition to activating NK_3 tachykinin receptors, neurokinin B can also bind to NK_1 and NK_2 tachykinin receptors (Table 2). Consequently to determine whether the excitation caused by neurokinin B was mediated by activation of NK_1 or NK_2 tachykinin receptors the ability of selective non-peptide antagonists to block these effects was studied. However, neither the tachykinin NK_1 antagonist CP 99,994 (1 μM for 30 min) or the NK_2 antagonist SR 48968 (300 nM for 30 min) blocked the effects of a submaximally effective concentration of neurokinin B (100 nM; Fig. 2; n = 4). Like neurokinin B,

Table 1
Potency of tachykinin agonists and antagonist affinity for receptors on presumed dopamine neurones in rat brain slices through the ventral tegmental area

Agonists	pEC_{50}	EC_{50} (nM)	n
Senktide	8.38 ± 0.14	4	6
Neurokinin B	7.49 ± 0.11	32	4
[β-Ala^8]NKA-(4–10)	6.37 ± 0.18	427	3
Substance P-O-methyl ester	< 6.5	> 300	10
GR 73632	< 6	> 1000	5
Antagonists	pK_A	K_A (nM)	n
CP 99,994	< 6	> 1000	4
SR 48968	< 6.5	> 300	4

Antagonist affinities were estimated using neurokinin B as the agonist.

the putative NK_2 selective receptor agonist [β-Ala^8]neurokinin A-(4–10) can also activate NK_3 tachykinin receptors at high concentrations (Table 2). To determine whether the effects of [β-Ala^8]neurokinin A-(4–10) were mediated by NK_2 tachykinin receptors the ability of SR 48968 to antagonise the responses to this agonist was examined. However, in three cells the excitation caused by [β-Ala^8]neurokinin A-(4–10) (3 μM) was not blocked by 30 min pretreatment with 300 nM SR 48968 (86 ± 7% of control).

Table 2
Selectivity of tachykinin receptor ligands (estimated from the published literature – see below) used in the present study for NK_1, NK_2 and NK_3 receptor subtypes

Ligand	Affinity or EC_{50} (nM)		
	NK_1	NK_2	NK_3
NK_1 preferring			
Substance P	2	2200	18000
Substance P-O-methyl ester	13	10000	> 10000
GR 73632	4	960	> 1000
CP 99,994	0.2	> 10000	> 10000
NK_2 preferring			
Neurokinin A	16	3	1300
[β-Ala^8]NKA-(4–10)	260	120	980
SR48968	> 5000	0.5	> 5000
NK_3 preferring			
Neurokinin B	70	25	4
Senktide	> 10000	> 10000	18

Values are compiled simply to compare the approximate subtype selectivity for each ligand. Data are binding affinities for rat tachykinin receptors where available (Cascieri et al., 1992; Emonds-Alt et al., 1992; Gether et al., 1993; Humpel and Saria, 1993), however data for [β-Ala^8]NKA-(4–10) (Rovero et al., 1989) and GR 73632 (Hagan et al., 1991) are from functional assays, and the affinity of CP 99,994 is for human tachykinin receptors (McLean et al., 1993; see text). The affinity of CP 99,994 for rat tachykinin NK_1 receptors is several-fold lower than that for human tachykinin NK_1 receptors but was used as the preferred antagonist in the present study because it lacks non-specific effects on ion channels (see text).

4. Discussion

Activation of tachykinin receptors on midbrain dopamine neurones will influence the release of dopamine in brain regions innervated by these cells. Mesolimbic dopamine neurones of the ventral tegmental area provide a major projection pathway to the nucleus accumbens and prefrontal cortex. In particular it has been hypothesised that hyperactivity of this pathway in man may underlie some forms of psychosis (reviewed in Goldstein and Deutch, 1992; Reynolds, 1992). Although the role of tachykinin receptors in psychiatric illness is unknown it has been suggested that antagonists of NK_1 tachykinin receptors may be useful therapeutic agents (Elliot et al., 1992). In view of the potential clinical significance of this proposal and because different studies report conflicting results concerning the relative importance of different tachykinin receptor subtypes in the ventral tegmental area, this study was carried out to determine the pharmacology of the tachykinin receptors that mediate the excitations caused by the exogenous application of tachykinins in this region of rat brain. These data demonstrate that the pharmacology of tachykinin receptors on rat ventral tegmental area neurones is consistent with the activation of NK_3 and not NK_1 or NK_2 tachykinin receptor subtypes.

4.1. Comparison with other studies

Neurokinin B is the endogenous ligand for NK_3 tachykinin receptors, but like other agonists can also

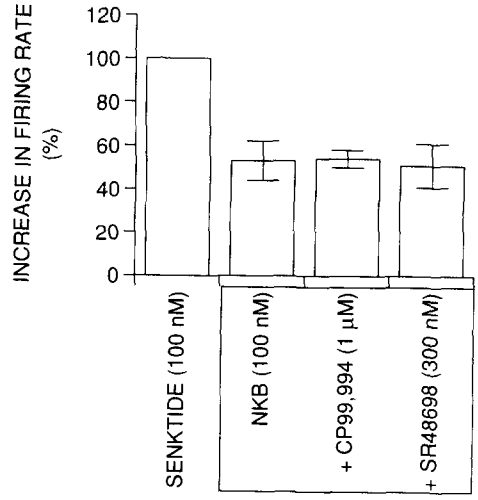

Fig. 2. Effect of tachykinin NK_1 (CP 99,994) and NK_2 (SR 48968) antagonists on the increase in excitability caused by the exogenous applicaton of a submaximally effective concentration of neurokinin B (100 nM). The increase in firing caused by neurokinin B was unaffected by pre-incubation with either CP 99,994 (1 μM) or SR 48968 (300 nM). Data are normalised to the maximal increase in firing rate caused by 100 nM senktide in each cell and represent the means ± S.E.M. of four cells.

activate more than one tachykinin receptor subtype (Table 2). In contrast the synthetic peptide, senktide, is one of the most selective ligands for NK_3 tachykinin receptors that has been described. [^{3}H]Senktide binds to several brain regions especially within frontal cortex, the medial but not dorsal raphe nuclei, hippocampus, substantia nigra and ventral tegmental area (Dam et al., 1990; Stoessl and Hill, 1990). Fibres immunoreactive to neurokinin B, the endogenous NK_3 tachykinin receptor ligand, are also widespread in the brain and are found in both the ventral tegmental area and substantia nigra (Marksteiner et al., 1992). In the present study both neurokinin B and senktide were potent and efficacious agonists in ventral tegmental area. However, in contrast to the comparable binding affinities of these ligands for clonal rat NK_3 tachykinin receptors the functional potency of senktide was 10-fold greater than that of neurokinin B (Table 2). The potency of an agonist is dependent not only upon its affinity for receptors but also upon its intrinsic activity and the receptor reserve (Kenakin, 1993), although it was not possible to determine whether differences in intrinsic activity could account for the greater potency of senktide in this study.

As in the ventral tegmental area, previous studies have also shown that senktide excites most substantia nigra neurones (Keegan et al., 1992; however c.f. Overton et al., 1992). In agreement with previous in vitro studies, senktide elicits behavioural effects concordant with the stimulation of midbrain dopamine neurones when applied directly to either the ventral tegmental area or substantia nigra (Stoessl et al., 1991). In the present study, exogenously applied neurokinin B also potently excited ventral tegmental area neurones, and consistent with the effects of neurokinin B being mediated by direct activation of NK_3 receptors, responses to this agonist were not blocked by selective non-peptide antagonists for NK_1 (CP 99,994) or NK_2 (SR 48968) receptors. Several non-peptide tachykinin NK_1 receptor antagonists, including CP 96,345 (Beresford et al., 1991) and CP 99,994 (McLean et al., 1992), have higher affinity for human and guinea-pig NK_1 receptors than those of the rat. Consequently in the present study the concentration of CP 99,994 used was chosen to be appropriate for its functional affinity for rat NK_1 receptors (ca. 300 nM; unpublished observations), and the effects of selective tachykinin NK_1 receptor agonists were also examined. Substance P, like neurokinin B, can also act upon more than one tachykinin receptor subtype (Table 2). In contrast the synthetic peptides substance P-O-methyl ester and GR 73632 have much greater selectivity for NK_1 over NK_2 receptors and were thus used as the preferred agonists to test for the presence of NK_1 tachykinin receptors in the ventral tegmental area. However, no effects of either agonist were observed on presumed dopamine neurones in this study. Consequently few if any NK_1 receptors were present on these cells. Several in vivo studies have also reported that only a minority of neurones in substantia nigra pars compacta respond to those tachykinin receptor agonists that act on NK_1 receptors (Pinnock and Dray, 1982; Keegan et al., 1992; Overton et al., 1992). Consistent with the absence of NK_1 receptors on dopamine neurones within the substantia nigra some behavioural studies have shown that tachykinin NK_1 receptor agonists do not stimulate locomotor activity when injected in this brain region (Stoessl et al., 1991; however see also Elliot et al., 1991). The grooming behaviour observed following central administration of NK_1 receptor agonists (Stoessl et al., 1991) may simply be a consequence of the inevitable diffusion of drugs away from the injection site (e.g. Humpel et al., 1991), as this behaviour is observed when tachykinin NK_1 receptor agonists are applied directly to the substantia nigra pars reticulata (Kelly and Iversen, 1978). Alternatively these behaviours may be a consequence of effects upon non-dopamine-containing neurones within the substantia nigra (e.g. Lacey et al., 1989), or upon afferent projections to this brain region.

Although no effects of selective tachykinin NK_1 receptor agonists were observed on presumed dopamine neurones of the ventral tegmental area in this in vitro and in other in vivo studies (Stoessl et al., 1991), at least one group has reported effects of tachykinin NK_1 receptor ligands in this tissue (Overton et al., 1992; Elliot et al., 1992). These conflicting results may partly relate to selectivity of some of the ligands tested, the experimental methodology, or alternatively to differences between the strains of animals used, however no gross differences in the distribution of tachykinin receptors between rat strains have been reported.

A third tachykinin receptor subtype, NK_2, is also found in both central nervous system and peripheral tissues. NK_2 tachykinin receptors are preferentially activated by the endogenous tachykinin neurokinin A, and are localised extensively within peripheral tissues but have only a restricted distribution within the brain (see Humpel and Saria, 1993). There are many conflicting studies concerning the effects of NK_2 receptor agonists in the central nervous system, some of which may simply relate to the non-selective effects of ligands on other tachykinin receptor subtypes. Indeed Kalivas et al. (1985), have shown that neurokinin A is ten-fold more potent than substance P at inducing locomotor activity in rat ventral tegmental area, however these effects may simply be due to the direct activation of NK_3 tachykinin receptors to which neurokinin A binds with higher affinity than does substance P (e.g. Gether et al., 1993; Table 2). Other more selective tachykinin NK_2 receptor agonists include GR 64349 (Hagan et al., 1991) and β-[Ala^8]neurokinin A-(4–10) (Rovero et

al., 1989). In the substantia nigra micromolar concentrations of β-[Ala^8]neurokinin A-(4–10) were found to be inactive (Keegan et al., 1992) whereas GR 64349 has been reported to increase the excitability of both substantia nigra and ventral tegmental area neurones (Overton et al., 1992). In the present study β-[Ala^8]neurokinin A-(4–10) was also found to excite neurones in the ventral tegmental area. However, because this ligand also has affinity for NK_1 and NK_3 tachykinin receptors it was important to determine whether these effects were specific to NK_2 receptors. SR 48968, a selective non-peptide antagonist of NK_2 tachykinin receptors (Emonds-Alt et al., 1992), did not block responses to β-[Ala^8]neurokinin A-(4–10) in this tissue and consequently these data were consistent with the direct activation of NK_3 tachykinin receptors. Confirmation of this hypothesis requires a selective antagonist for rat NK_3 tachykinin receptors and these ligands have yet to be described.

4.2. Functional implications

The source of neurokinin B fibres in the ventral tegmental area is not clear (reviewed in Kalivas, 1993; Angulo and McEwen, 1994), but may involve the afferent 5-hydroxytryptamine-containing fibres of the medial habenula and raphe (e.g. Marksteiner et al., 1992). 5-Hydroxytryptamine-containing fibres in the medial habenula also have NK_3 tachykinin receptors on their cell bodies and activation of these receptors stimulates locomotor responses that can be blocked by dopamine receptor antagonists, presumably by an effect in the striatum.

The localisation of both neurokinins and tachykinin receptor subtypes within the striatum is also heterogeneous. Neurokinin B immunoreactive fibre's are co-localised within GABA-containing neurones that project to the pallidum but not the substantia nigra pars reticulata, whereas substance P- containing neurones project principally to the substantia nigra pars reticulata (Burgunder and Young, 1989). Activation of tachykinin receptors in the striatum modulates the release of neurotransmitters including acetylcholine and dopamine (Tremblay et al., 1992). Interestingly in that study the release of dopamine by [Pro^7]neurokinin B (NK_3) was partly insensitive to tetrodotoxin suggesting a presynaptic localisation of NK_3 tachykinin receptors. This is further supported by the finding that stimulation of acetylcholine release by neurokinin B in rat striatum is reduced by both tetrodotoxin and by lesions of the nigrostriatal pathway (Arenas et al., 1991), and is consistent with the presence of NK_3 tachykinin receptors on dopamine cell bodies of the striatonigral (Keegan et al., 1992) and mesolimbic pathways (this study). However, it has recently been reported that 6-hydroxydopamine lesions of the medial forebrain bundle in rat brain significantly decreases senktide binding in the substantia nigra but not in the ventral tegmental area (Stoessl, 1994). These results indicate that a substantial proportion of the NK_3 binding sites in the ventral tegmental area is not located on dopamine cell bodies. Consequently, activation of only a minority of the total NK_3 receptors by senktide may be necessary for the increase in neuronal excitability, or alternatively this increase may be mediated by a population of presynaptic NK_3 receptors that modulate the release of excitatory neurotransmitters within the ventral tegmental area.

In conclusion, this study indicates that the principal tachykinin receptor subtype regulating dopamine cell excitability within the rat ventral tegmental area is of the NK_3 and not the NK_1 or NK_2 subtype. Although these data suggest that NK_3 tachykinin receptors mediate the principal excitatory influence of tachykinins on mesolimbic dopamine neurones, the role of receptors on afferent projections to the ventral tegmental area and the relative tone of neuropeptide-containing fibres may also have a significant influence over their function.

Acknowledgements

The authors are grateful to Jeffrey J. Hale and Eileen Seward for the synthesis of SR 48968 and CP 99,994.

References

Angulo, J.A. and B.S. McEwen, 1994, Molecular aspects of neuropeptide regulation and function in the corpus striatum and nucleus accumbens, Brain Res. Rev. 19, 1.

Arenas, E., J. Alberch, E. Perez-Navarro, C. Solsona and J. Marsal, 1991, Neurokinin receptors differentially mediate endogenous acetylcholine release evoked by tachykinins in the neostriatum, J. Neurosci. 11(8), 2332.

Beresford, I.J.M., P.J. Birch, R.M. Hagan and S.J. Ireland, 1991, Investigation into differences in tachykinin NK_1 receptors between and within species using a peptide and non-peptide NK_1 receptor antagonist, Br. J. Pharmacol. 104, 292.

Bowery, B., L.A. Rothwell and G.R. Seabrook, 1994, Comparison betwen the pharmacology of dopamine receptors mediating the inhibition of cell firing in brain slices through the rat substantia nigra pars compacta and ventral tegmental area, Br. J. Pharmacol. 112(3), 873.

Burgunder, J.M. and W.S. Young, 1989, Distribution, projection, and dopaminergic regulation of the neurokinin B mRNA-containing neurones of the rat caudate-putamen, Neuroscience 32, 323.

Dam, T.-V., E. Escher and R. Quirion, 1990, Visualisation of neurokinin-3 receptor sites in rat brain using the highly selective ligand [^{3}H]senktide, Brain Res. 506, 175.

Davies, J. and A. Dray, 1976, Substance P in the substantia nigra, Brain Res. 107, 623.

Elliot, P.J., G.S. Mason, M. Stephens-Smith and R.M. Hagan, 1991, Behavioural and biochemical responses following activation of

midbrain dopamine pathways by receptor selective neurokinin agonists, Neuropeptides 19, 119.

Elliot, P.J., G.S. Mason, E.A. Graham, M.P. Turpin and R.M. Hagan, 1992, Modulation of the rat mesolimbic dopamine pathway by neurokinins, Behav. Brain Res. 51, 77.

Emonds-Alt, X., P. Vilain, P. Goulaouic, V. Proietto, D. Van Broeck, C. Advenier, E. Naline, G. Neliat, G. Le Fur and J.C. Breliere, 1992, A potent and selective non-peptide antagonist of the neurokinin A (NK_2 receptor), Life Sci. 50, 101.

Gerfen, C.R., 1991, Substance P (neurokinin-1) receptor mRNA is selectively expressed in cholinergic neurons in the striatum and basal forebrain, Brain Res. 556, 165.

Gether, U., T.E. Johansen and T.W. Schwartz, 1993, Chimeric NK_1 (substance P)/NK_3 (neurokinin B) receptors, J. Biol. Chem. 268(11), 7893.

Goldstein, M. and A.Y. Deutch, 1992, Dopaminergic mechanisms in the pathogenesis of schizophrenia, FASEB J. 6, 2413.

Guyenet, P.G. and G.K. Aghajanian, 1977, Excitation of neurones in the nucleus locus coeruleus by substance P and related peptides, Brain Res. 136, 178.

Hagan, R.M., S.J. Ireland, C.C. Jordan, I.J.M. Beresford, M.J. Deal and P. Ward, 1991, Receptor-selective, peptidase-resistant agonists at neurokinin NK_1 and NK_2 receptors: new tools for investigating neurokinin function, Neuropeptides 19, 127.

Humpel, C. and A. Saria, 1993, Characterisation of neurokinin binding sites in rat brain membranes using highly selective ligands, Neuropeptides 25(1), 65.

Humpel, C., A. Saria and D. Regoli, 1991, Injection of tachykinins and selective neurokinin receptor ligands into the substantia nigra reticulata increases striatal dopamine and 5-hydroxytryptamine metabolism, Eur. J. Pharmacol. 195, 107.

Innis, R.B., R. Andrade and G.K. Aghajanian, 1985, Substance K excites dopaminergic and non-dopaminergic neurones in rat substantia nigra, Brain Res. 335, 381.

Iversen, L.L., A.T. Mcknight, A.C. Foster, S.C. Young and B.J. Williams, 1990, Pharmacology of the tachykinin system, in: Neuropeptides and Their Receptors, eds. T.W. Schwartz, L.M. Hilsted, and J.F. Rehfeld (Munksgaard, Copenhagen) p. 363.

Johnson, S.W. and R.A. North, 1992, Two types of neurone in the rat ventral tegmental area and their synaptic inputs, J. Physiol. (London) 450, 455.

Kalivas, P.W., 1993, Neurotransmitter regulation of dopamine neurons in the ventral tegmental area, Brain Res. Rev. 18, 75.

Kalivas, P.W., A.Y. Deutch, J.E. Maggio, P.W. Mantyh, and R.H. Roth, 1985, Substance K and substance P in the ventral tegmental area, Neuroscience Lett. 57, 241.

Keegan, K.D., G. Woodruff and R.D. Pinnock, 1992, The selective NK_3 receptor agonist senktide excites a subpopulation of dopamine-sensitive neurones in the rat substantia nigra pars compacta in vitro, Br. J. Pharmacol. 105, 3.

Kelly, A.E. and S.D. Iversen, 1978, Behavioural response to bilateral injections of substance P into the substantia nigra of rat, Brain Res. 158, 474.

Kenakin, T., 1993, Pharmacologic Analysis of Drug-Receptor Interaction (Raven Press, New York) p. 221.

Lacey, M.G., N.B. Mercuri and R.A. North, 1988, On the potassium conductance increase activated by $GABA_B$ and dopamine D_2 receptors in rat substantia nigra neurones, J. Physiol. (London) 401, 437.

Lacey, M.G., N.B. Mercuri and R.A. North, 1989, Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids, J. Neurosci. 9(4), 1233.

Marksteiner, J., G. Sperk and J.E. Krause, 1992, Distribution of neurons expressing neurokinin B in the rat brain: immunohistochemical and in situ hybridisation, J. Comp. Neurol. 317, 341.

Mantyh, P.W., T. Gates, C.R. Mantyh and J.E. Maggio, 1989, Autoradiographic localisation and characterisation of tachykinin receptor binding sites in the rat brain and peripheral tissues, J. Neuroscience 9(1), 258.

McLean, S., A. Ganong, P.A. Seymour, R.M. Snider, M.C. Desai, T. Rosen, D.K. Bryce, K.P. Longo, L.S. Reynolds, G. Robinson, A.W. Schmidt, C. Siok and J. Heym, 1993, Pharmacology of CP 99,994: a nonpeptide antagonist of the tachykinin NK_1 receptor, J. Pharmacol. Exp. Ther. 267, 472.

Norris, S.K., P.R. Boden and G.N. Woodruff, 1993, Agonists selective for tachykinin NK_1 and NK_3 receptors excite subpopulations of neuron in the rat medial habenula nucleus in vitro, Eur. J. Pharmacol. 234, 223.

Overton, P., P.J. Elliot, R.M. Hagan and D. Clark, 1992, Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat, Eur. J. Pharmacol. 213, 165.

Paxinos, G. and C. Watson, 1944, The Rat Brain in Stereotaxic Coordinates (Academic Press, London).

Pinnock, R.D., 1983, Sensitivity of compacta neurones in the rat substantia nigra slice to dopamine agonists, Eur. J. Pharmacol. 96, 269.

Pinnock, R.D. and A. Dray, 1982, Differential sensitivity of presumed dopaminergic and non-dopaminergic neurones in rat substantia nigra to electrophoretically applied substance P, Neurosci. Lett. 29, 153.

Pomeroy, S.L. and M.M. Behbehani, 1980, Response of nucleus raphe magnus neurons to iontophoretically applied substance P in rats, Brain Res. 202, 464.

Reynolds, G.P., 1992, Developments in the drug treatment of schizophrenia, Trends Pharmacol. Sci. 13, 116.

Rovero, P., V. Pestillini, V. Patacchini, S. Giuliani, P. Santicioli, C.A. Maggi and A. Giachetti, 1989, A potent and selective agonist for NK_2 tachykinin receptor, Peptides 10(3), 593.

Seabrook, G.R., M.J. Main, Z. Razzaque and J. Longmore, 1993, Differences in the effects of tachykinin NK_1 receptor antagonists: neuronal versus smooth muscle tissues, Eur. J. Pharmacol. 250, 125.

Stoessl, A.J., 1994, Localisation of striatal and nigral tachykinin receptors in the rat, Brain Res. 646, 13.

Stoessl, A.J. and D.R. Hill, 1990, Autoradiographic visualisation of NK_3 tachykinin binding sites in the rat brain utilising [^{3}H]senktide, Brain Res. 534, 1.

Stoessl, A.L., E. Szczutkowski, B. Glenn and I. Watson, 1991, Behavioural effects of selective tachykinin agonists in midbrain dopamine neurones, Brain Res. 565, 254.

Tremblay, L., M.-L. Kemel, M. Desban, C. Gauchy and J. Glowinski, 1992, Distinct presynaptic control of dopamine release in striosomal-matrix-enriched areas of the rat striatum by selective agonists of NK_1, NK_2 and NK_3 tachykinin receptors, Proc. Natl. Acad. Sci. USA 89, 11214.