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Substance P-(1–7) and substance P-(5–11) locally modulate dopamine release in rat striatum

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Abstract

The effects of substance P, substance P-(1–7) and substance P-(5–11) on endogenous dopamine outflow in rat striatal slices were investigated. The dose-response curves (0.01 nM to 10 μM) were bell-shaped, with significant increases at 0.1 and 1 nM but with no effect at higher concentrations. The tachykinin NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]substance P, significantly increased dopamine outflow at 10 and 100 nM. The effects of substance P or substance P-(5–11) and 25 mM KCl were additive. A negative interaction was observed with substance P-(1–7) and K^+. The increase in dopamine outflow elicited by 1 nM substance P and substance P-(5–11) was reversed by the tachykinin NK_1 receptor antagonist WIN 51,708 (17β-hydroxy-17α-ethynyl-5α-androstano[3,2-*b*]pyrimido[1,2-*a*]benzimidazole) (25 and 250 nM), whereas only partial reversal was observed for the effect of substance P-(1–7). These results show that substance P fragments locally modulate striatal dopamine outflow and the mechanisms underlying this modulation may differ between N-and C-terminal fragments.

Keywords: Substance P fragment; Striatum; Dopamine outflow; Tachykinin receptor

1. Introduction

Numerous studies have documented the presence of substance P in the basal ganglia, where the peptide is localized in a major population of striatal projection neurones whose terminals are mainly located in the substantia nigra (review in Gerfen, 1992). Substance P immunoreactivity has also been detected in nerve terminals in the striatum, their origin being the axon collaterals of the projection neurones (Bolam et al., 1983; Kawaguchi et al., 1989). The contribution of substance P-producing intrinsic striatal neurones to this immunoreactivity is thought to be much smaller than that of substance P-producing medium size spiny neurones. These observations suggest that the tachykinin could play a role in both striatum and substantia nigra, where evidence of its release has been obtained (Lindefors et al., 1989). Several reports have suggested that one of the roles of substance P is to act as a modulator of dopamine release from nigrostriatal dopaminergic neurones. Thus, substance P applied intranigrally stimulates dopamine release in the striatum (Reid et al., 1990). Local modulation of dopamine release in the striatum has also been shown in studies using substance P and monitoring the release of radiolabelled dopamine from striatal slices (Petit and Glowinski, 1986).

Substance P can be cleaved by several peptidases, such as a phosphoramidon-sensitive neutral endopeptidase (EC 3.4.24.11; 'enkephalinase') (Skidgel et al., 1984), angiotensin-converting enzyme (EC 3.4.15.1) (Yokosawa et al., 1983), a substance P-degrading peptidase (Lee et al., 1981) and a post-proline cleaving enzyme (Blumberg et al., 1980). Some of the fragments generated by enzymatic hydrolysis, such as substance P-(1–7) or C-terminal fragments, have been shown to be present in significant concentrations in the central nervous system, and in particular in the striatum (Sakurada et al., 1985). Increasing evidence suggests that such fragments may exert effects which are similar to, or different from, the effects of the parent peptide. This has been demonstrated in a variety of experimental models and has also been reported in studies on dopamine release. It has been shown that substance

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P-(1–7) and substance P-(6–11) modulate striatal dopamine release when applied intranigrally (Reid et al., 1990). However, it is not known yet if after substance P release from axon collaterals of striatonigral neurones and enzymatic hydrolysis, substance P fragments could also exert neuromodulatory effects in the striatum. Therefore, in the present study we have investigated whether substance P N-and C-terminal fragments could modulate spontaneous and stimulated endogenous dopamine release after local application in rat striatal slices. The peptide fragments chosen were substance P-(1–7) and substance P-(5–11). Substance P and the selective tachykinin NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]substance P were tested in parallel.

2. Materials and methods

2.1. Preparation of slices

Male Wistar rats (200–250 g) were killed by stunning and decapitation. The brain was rinsed in ice-cold Krebs buffer and coronal striatal slices (approximate thickness 400–500 μm) were cut over ice and washed in fresh buffer (2 × 20 ml for 4 hemistriata).

2.2. Monitoring dopamine outflow in striatal slices

Dopamine outflow was monitored in striatal slices using either a superfusion or a sequential incubation method. Changes in endogenous dopamine outflow can be quantified using either method with similar results, as suggested in a recent study (Khan et al., 1995). The effects of various agents (e.g. high K^+ concentrations, veratridine, 4-aminopyridine, tetrodotoxin) are comparable in the two models. Sequential incubation can be used for greater efficiency (i.e. less samples without the inconvenient dilution of analytes in the samples), although the temporal resolution of the analysis is reduced. In the superfusion method, slices (2 per chamber) were superfused with buffer (0.2 ml/min) for an equilibration period of 60 min prior to sample collection. Subsequently, slices were superfused with buffer in the absence (basal outflow) or presence of tested substances. Basal outflow was defined as the average dopamine concentration measured in the first 2 collected samples. Samples were collected every 2 min into tubes containing ascorbic acid (final concentration 0.1 mM), phosphoric acid (final concentration 0.1 M) and dihydroxybenzylamine (final concentration 23 nM). At the end of the experiment the slices were homogenized by sonication in perchloric acid (0.1 N) containing dihydroxybenzylamine (final concentration 23 nM). The homogenate was centrifuged (7000 × g for 10 min) and the supernatant was analyzed by high performance liquid chromatography. The protein content of the pellet was determined using Lowry's method (Lowry et al., 1951) and bovine serum albumin as a standard. In the sequential incubation method, slices were placed on tissue holders which were transferred between tubes. After a 60 min equilibration in buffer (200 ml for 4 hemistriata), 2 slices were placed on each tissue holder and lowered into tubes containing 1 ml of buffer. The tissue was incubated for 5 min periods in buffer in the absence (basal outflow) or presence of tested substances. Basal outflow was defined as the average dopamine concentration measured in the first two tubes. After transfer of the slices to a new tube, the previous tube was placed on ice and 500 μl were transferred to a tube containing ascorbic acid, phosphoric acid and dihydroxybenzylamine (final concentrations as above). After centrifugation (7000 × g for 10 min) the supernatant was kept for high performance liquid chromatography analysis. Slices were processed as described above.

2.3. Composition of buffer and agents tested

The buffer contained (mM): NaCl 121, KCl 1.87, KH_2PO_4 1.17, $MgSO_4$ 1.17, $CaCl_2$ 1.22, $NaHCO_3$ 25, glucose 11.1. It was saturated with 95% O_2 and 5% CO_2 and the pH was adjusted to 7.4. The various substances tested were added to the buffer after collection of the first two samples. 25 mM KCl was applied as a 1 min pulse. When the KCl concentration was increased, the NaCl concentration was decreased accordingly, to preserve isotonicity. Slices were exposed to substance P, substance P-(1–7), substance P-(5–11) and [Sar^9,$Met(O_2)^{11}$]substance P for 10 min. WIN 51,708 (17β-hydroxy-17α-ethynyl-5α-androstano[3,2-*b*]pyrimido[1,2-*a*]benzimidazole) was added to the buffer after the first two samples and was maintained throughout the experiment. When the peptides were studied in the presence of 25 mM KCl, the depolarizing pulse was applied during the first minute of exposure to peptide.

2.4. High performance liquid chromatography and analysis of data

The concentration of dopamine was measured by high performance liquid chromatography coupled with electrochemical detection. Dihydroxybenzylamine was used as an internal standard. The analytical column was an Ultrasphere ODS reversed phase column (75 × 4.6 mm i.d., 3 μm) with a precolumn packed with Co-Pell ODS (20 × 2 mm i.d., 30–40 μm). The eluent consisted of 0.1 M NaH_2PO_4, octane sulphonic acid (0.025% w/v), methanol (10% v/v), pH 3.9, pumped at 1 ml/min. Peaks were detected using a Coulochem II model 5100 detector operated in the redox mode, with porous graphite electrodes set at +0.2 V and −0.25 V

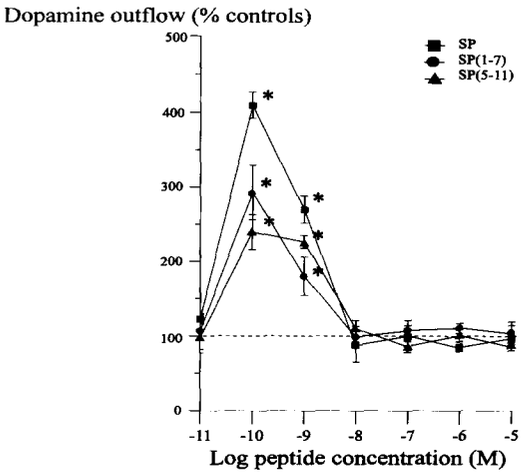

Fig. 1. Striatal slices were exposed to increasing concentrations of substance P, substance P-(1–7) and substance P-(5–11). Results are expressed as percentages of controls and represent means ± S.E.M. of 5–6 experimental values. In superfusion experiments typical basal outflow in controls was 1.32 ± 0.06 pmol dopamine/ml/mg protein ($n = 84$ cumulated controls). * $P < 0.05$ vs. the respective controls.

vs. Pd. The reduction signal was used for quantification. Dopamine outflow was expressed as pmol/ml/mg protein. Changes in dopamine outflow due to treatments were expressed as a percentage of baseline. The baseline was calculated by extrapolating the average basal outflow (measured in the first 2 samples before drug) for the duration of the effect of drugs (i.e. defining the area under the curve), and represented the control value. Results were expressed as means ± S.E.M. and were analyzed by one-way or two-way analysis of variance and Bonferroni's test for multiple comparisons.

2.5. Reagents

Dopamine, dihydroxybenzylamine and substance P-(5–11) were from Sigma Chemicals (Poole, Dorset, UK). Substance P-(1–7) and substance P were from Calbiochem-Novabiochem (Beeston, Nottingham, UK). [Sar^9,Met(O$_2$)^{11}]substance P and WIN 51,708 were from Research Biochemicals International (Semat, St. Albans, Hertfordshire, UK). All other reagents were from Fisons Scientific (Loughborough, Leicestershire, UK) and BDH Laboratory Supplies (Lutterworth, Leicestershire, UK).

3. Results

3.1. Effects of substance P, substance P-(1–7) and substance P-(5–11) on spontaneous dopamine outflow

Fig. 1 shows that increasing concentrations of substance P, substance P-(1–7) and substance P-(5–11) significantly modified spontaneous dopamine outflow ($F(7,76) = 90.5$, $P < 0.05$; $F(7,76) = 10.5$, $P < 0.05$; $F(7,76) = 29.7$, $P < 0.05$, for substance P, substance P-(1–7) and substance P-(5–11), respectively). The effects of the three peptides followed a bell-shaped curve, with a significant increase in dopamine outflow at 0.1 nM and a smaller effect at 1 nM, whereas concentrations lower than 0.1 nM or higher than 1 nM failed to modify dopamine release. When tested at concentrations of 1 nM, 10 nM, 100 nM and 1 μM, the tachykinin NK$_1$ receptor agonist [Sar^9,Met(O$_2$)^{11}]substance P induced the following increase in dopamine outflow (% controls): 195 ± 46, 349 ± 71 ($P < 0.05$ vs. controls), 259 ± 58 ($P < 0.05$ vs. controls) and 163 ± 41, respectively.

3.2. Effects of substance P, substance P-(1–7) and substance P-(5–11) on K$^+$-induced dopamine overflow

The exposure of slices to 25 mM KCl induced a significant increase in dopamine outflow (% controls): 207 ± 16 ($P < 0.05$ vs. controls). When the pulse of 25 mM KCl was applied in the presence of peptides, differences were observed between substance P, substance P-(1–7) and substance P-(5–11). The effects of 25 mM KCl and 1 nM substance P were additive (% controls): 207 ± 31 ($P < 0.05$ vs. controls) with peptide only and 409 ± 82 ($P < 0.05$ vs. controls) in the presence of K$^+$. A similar additivity was seen with substance P-(5–11) (% controls): 196 ± 19 ($P < 0.05$ vs. controls) with substance P-(5–11) only and 310 ± 31

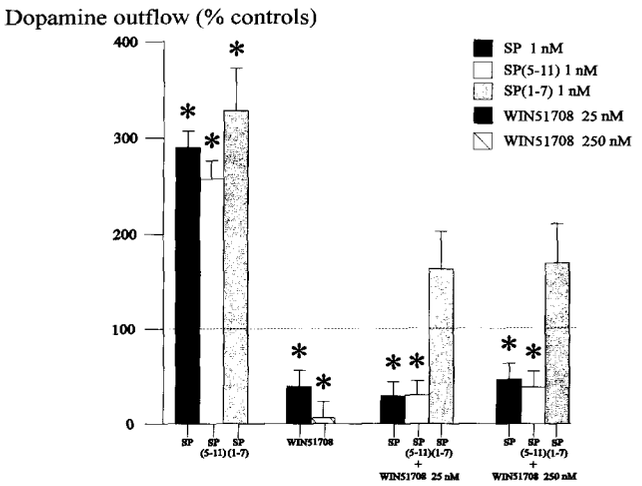

Fig. 2. Striatal slices were exposed to 1 nM substance P, substance P-(1–7) and substance P-(5–11) in the absence or presence of WIN 51,708 (25 and 250 nM). Results are expressed as percentages of controls and represent means ± S.E.M. of 5–6 experimental values. In superfusion experiments typical basal outflow in controls was 1.32 ± 0.06 pmol dopamine/ml/mg protein ($n = 84$ cumulated controls). * $P < 0.05$ vs. the respective controls.

($P < 0.05$ vs. controls) when the K^+ pulse was applied. In contrast, a negative interaction between the response to 1 nM substance P-(1–7) and 25 mM KCl was observed ($F(1,32) = 9.85$, $P < 0.01$). Thus, the increase in dopamine outflow compared to controls was 286 ± 69 in the presence of substance P-(1–7), and 253 ± 10 in the presence of peptide and K^+ ($P < 0.05$ vs. controls).

3.3. Effect of WIN 51,708 on dopamine overflow induced by substance P, substance P-(1–7) and substance P-(5–11)

As shown in Fig. 2, the tachykinin NK_1 receptor antagonist WIN 51,708 had an intrinsic effect and dose-dependently decreased dopamine outflow, when tested at 25 and 250 nM ($F(2,19) = 34.4$, $P < 0.001$). The antagonist completely reversed the effects of 1 nM substance P and substance P-(5–11), whereas partial reversal was apparent for substance P-(1–7)-induced dopamine overflow (Fig. 2).

4. Discussion

The main finding of this study is that N-and C-terminal fragments of substance P modulate locally dopamine outflow in the striatum, and interestingly, the pattern of modulation is different from that induced after intranigral application (Reid et al., 1990). We also confirm that substance P can modulate striatal dopamine outflow locally, as suggested previously using tritiated dopamine (Petit and Glowinski, 1986). In contrast with its fragments, the effects of the parent peptide applied locally show a profile similar to that reported after nigral administration (Reid et al., 1990).

In our study, substance P-(1–7) and substance P-(5–11) induced a significant increase in striatal dopamine outflow. In contrast, Reid et al. (1990) reported that substance P-(6–11) induced a decrease in striatal dopamine release, whereas substance P-(1–7) decreased dopamine release at low concentrations but increased it at high concentrations. The nature of the receptor(s) involved in the effects observed in the striatum and the substantia nigra may also differ. The effects induced by substance P-(6–11) injected in the substantia nigra were insensitive to a tachykinin NK_1 receptor antagonist (Reid et al., 1990), whereas in our study the effect of substance P-(5–11) was completely reversed in the presence of a selective tachykinin NK_1 receptor antagonist. The effects induced by substance P-(1–7) after nigral or striatal application also display different sensitivity to blockade of tachykinin NK_1 receptors. Only the decrease in striatal dopamine outflow elicited after intranigral application was sensitive to tachykinin NK_1 receptor antagonists, whereas the increase was not reversed by the same agents (Reid et al., 1990). In our model, the dopamine overflow elicited by substance P-(1–7) was diminished in the presence of a tachykinin NK_1 receptor antagonist. The clear reversal induced by WIN 51,708 suggests that in the striatum substance P and substance P-(5–11) elicit dopamine overflow by activation of tachykinin NK_1 receptors. The effect seen after exposure of tissue to a selective tachykinin NK_1 receptor agonist supports this explanation. The intrinsic effect of the tachykinin NK_1 receptor antagonist suggests that a tonic excitatory control may be exerted through tachykinin NK_1 receptors. The limited sensitivity of substance P-(1–7) to WIN 51,708 even at high concentrations of antagonist suggests that the N-terminal fragment might exert its action through a different neurokinin receptor subtype, or a complex mechanism, involving more than the activation of tachykinin NK_1 receptors. The existence of tachykinin NK_1 receptor subtypes has been suggested and evidence has also been obtained of existence of binding sites selective for substance P N-terminal fragments (Igwe et al., 1990). A different mechanism of action for substance P-(1–7) is also suggested by the negative interaction with K^+-induced dopamine overflow. This effect is reminiscent of the inhibitory action of substance P-(1–7) on substance P-induced dopamine overflow after nigral application (Reid et al., 1990). Such observations, also reported in other models (Skilling et al., 1990), have led to the suggestion that substance P-(1–7) provides an inhibitory feedback on the effects of the parent peptide. Although the effects of substance P and substance P-(1–7) on spontaneous dopamine outflow follow an identical pattern, it cannot be ruled out that during increased firing of dopaminergic cells, the (1–7) fragment could moderate the effects of substance P on the overflow of catecholamine. The bell-shaped curve observed with the peptides and the tachykinin NK_1 receptor agonist suggests that at high concentrations, endogenous or synthetic agents which stimulate tachykinin NK_1 receptors could trigger an inhibitory mechanism, which is responsible for a resulting lack of effect on dopamine outflow. Tachykinin NK_1 receptors are present in the striatum and are located on cholinergic and somatostatinergic neurones (Gerfen, 1991; Kaneko et al., 1993). This is in contrast with substantia nigra, where a mismatch is constantly found, i.e. the presence of high concentrations of substance P and the absence of tachykinin NK_1 receptors (Kaneko et al., 1993). Stimulation of striatal tachykinin receptors leads to increased acetylcholine release (Arenas et al., 1991), therefore the effect of peptide fragments may be underlied by a similar mechanism. One possible explanation of the biphasic curve is that acetylcholine may in turn activate an inhibitory circuit, mediated for example by striatal dynorphin (Gauchy et al., 1991). Another hypothesis is a direct inhibition of dopamine outflow

exerted through a non-tachykinin NK_1 receptor mechanism at higher doses.

The present study provides new evidence that peptide metabolites could exert intrinsic modulatory effects in the central nervous system. It also suggests the interesting possibility that different modulatory influences could be exerted on a given neurotransmitter pathway (e.g. dopaminergic in our case), when peptides originating in the same neuron are released by terminals or axon collaterals. The data highlight the possible complexity of the neuromodulation exerted by tachykinins. It is interesting to note that substance P has also been shown to stimulate striatal dopamine release after systemic administration (Boix et al., 1992b). In the same model, substance P fragments were devoid of effects after peripheral administration (Boix et al., 1992a). Finally, considering the heterogeneity of the striatum (Gerfen, 1992), differences in neuromodulation within this structure, for instance in striosomes versus striatal matrix, should also be investigated with biologically active peptide fragments.

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