 Substance P Depresses Excitatory Synaptic Transmission in the Nucleus Accumbens Through Dopaminergic and Purinergic Mechanisms

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Kombian, Samuel B., Kethireddy V. V. Ananthalakshmi, Subramanian S. Parvathy, and Wandikayi C. Matowe. Substance P depresses excitatory synaptic transmission in the nucleus accumbens through dopaminergic and purinergic mechanisms. J Neurophysiol 89: 728–737, 2003; 10.1152/jn.00854.2002. Substance P (SP) is an un-decapeptide that is co-localized with conventional transmitters in the nucleus accumbens (NAc). Its neurochemical and behavioral effects resemble those of cocaine and amphetamine. How SP accomplishes these effects is not known, partly because its cellular and synaptic effects are not well characterized. Using whole cell and nystatin-perforated patch recording in rat forebrain slices, we show here that SP, an excitatory neuropeptide, depresses evoked excitatory postsynaptic currents (EPSCs) and potentials (EPSPs) in NAc through interneuronal neurotransmitters. SP caused a partially reversible, dose-dependent decrease in evoked EPSCs. This effect was mimicked by a neurokinin-1 (NK1) receptor-selective agonist, [Sar9, Met(O2)11]-SP and blocked by a NK1 receptor-selective antagonist, L 732 138. In contrast to its effect on PPR, SP did not produce significant changes in the holding current, input resistance, EPSC decay rate (γ), and steady-state V-I curves of the recorded cells. The SP-induced synaptic depressions were prevented by dopamine receptor blockade using SCH23390 and haloperidol, but not by sulpiride. In addition, the SP-induced synaptic depression was blocked by an adenosine A1 receptor blocker 8-cyclopentyltheophylline (8-CPT) but not by the N-methyl-d-aspartate (NMDA) receptor antagonist d-APV. These data show that SP, by activating presynaptic NK1 receptors, depresses excitatory synaptic transmission indirectly by enhancing extracellular dopamine and adenosine levels. Since the cellular and synaptic effects of SP resemble those of cocaine and amphetamine, it may serve as an endogenous psychogenic peptide.

INTRODUCTION

The NAc is a forebrain structure located ventral to the neostriatum that has been implicated in complex behaviors such as drug-seeking behaviors and in the pathophysiology of psychiatric disorders (Koob and Bloom 1988; Swerdlow and Koob 1987). It is part of the mesolimbic dopamine system and receives dopaminergic inputs from the ventral tegmental area (VTA) and glutamatergic inputs from cortical and subcortical limbic areas (Bjorklund and Hokfelt 1983; Brog et al. 1993; Pennartz et al. 1994; Sesack et al. 1989). It is thought to serve as an interface where emotional events of limbic origin are converted into behavioral motor output. The majority of NAc cells (>90%) are medium spiny GABAergic projection neurons that exert a strong inhibitory influence onto neighboring accumbens neurons via an extensive network of local axon collaterals. In addition, a fraction of the remaining neurons that are GABAergic aspiny interneurons may contribute to inhibition through a feed forward action when activated by afferent excitatory neurons (Meredith 1999). The remaining aspiny interneurons are cholinergic (O’Donnell and Grace 1993; Pennartz and Kitai 1991; Pennartz et al. 1994).

Most of the major transmitters are co-localized with peptides (Fuxe et al. 1980; Jennes et al. 1982; Kalivas 1985b; Pickel et al. 1988; Uhl et al. 1977), which have been shown to influence their function (Huston and Hasenohrl 1995; Iversen 1982; Kalivas 1985a; Kalivas and Miller 1984a). One such peptide is SP, a tachykinin that is co-localized with GABA in the GABAergic projection neurons of the NAc (Napier et al. 1995), which produces biochemical and behavioral changes when injected into the NAc (Huston and Hasenohrl 1995; Iversen 1982; Kalivas and Miller 1984b; Schildelin et al. 1998), intracerebroventriculally (Krasnova et al. 2000), intraperitoneally (Boix et al. 1992a), or when the neurokinin receptors are disrupted (Murtra et al. 2000). Substance P-prefering receptors, the neurokinin1(NK1) receptors, are present in the NAc (Nakaya et al. 1994; Quirion et al. 1983) but are found mainly on the somatodendrites of aspiny cholinergic interneurons and on terminals in this region and rarely on the main, medium spiny GABAergic projection cells (Murtra et al. 2000; Pickel et al. 2000).

The main neurochemical effects of SP in the NAc appear to be an enhancement in the extracellular levels of dopamine and its metabolites (Boix et al. 1992a;b; Cador et al. 1989; Elliott et al. 1986b; Krasnova et al. 2000), as well as decreasing the extracellular levels of acetylcholine (Boix et al. 1994). Dopamine acts in the NAc to decrease both excitatory and inhibitory
synaptic transmission (Harvey and Lacey 1996; Nicola and Malenka 1997; Nicola et al. 1996). While SP, based on its neurochemical and behavioral effects, may be predicted to modulate excitatory synaptic transmission in a manner similar to cocaine and amphetamine (Nicola et al. 1996), it is not clear what the effect of SP is on synaptic transmission in this nucleus.

To understand the role of SP on the synaptic physiology of the NAc and its possible involvement in reward processes and the development of addiction to psychostimulant drugs, we tested the hypothesis that SP will modulate excitatory transmission in this nucleus. This study thus examined the effects of SP on evoked EPSCs/EPSPs and tested whether these were direct effects of the peptide or they were indirectly mediated through intermediate neuromodulators. The results of this study indicate that SP causes depression of evoked EPSCs/EPSPs by activating presynaptic NK1 receptors located on dopaminergic terminals to release DA. Dopamine, through an as yet not well-established mechanism, produces adenosine, which then causes the observed decrease in evoked EPSCs/EPSPs.

METHODS

All experiments in this study were carried out on rats obtained from the Kuwait University Animal Centre. International guidelines on humane handling of animals were followed throughout this study and the minimum number of animals necessary to produce the required results was used. Forebrain slices containing the nucleus accumbens and the cortex were generated using previously published techniques (Kombian and Malenka 1994). Briefly, male Sprague-Dawley rats (75–250 g) were anesthetized with halothane before decapitation. The brain was quickly removed from the rat and placed in ice-cold (4°C) artificial cerebrospinal fluid (ACSF) that was bubbled with 95% O2-5% CO2. The composition of the ACSF was (in mM) 126 NaCl, 2.5 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 18 NaHCO3, and 11 glucose. Parasagittal forebrain slices (350–400 μm thick) were cut in the ice-cold ACSF using tissue slicers [Electron Microscopy Sciences (OTS-4000) or Leica (VT 1000S)]. Slices were incubated in ACSF in the ice-cold ACSF using tissue slicers [Electron Microscopy Sciences – 3 ml/min (28°C)] or Leica (VT 1000S)]. Slices were incubated in ACSF using tissue slicers [Electron Microscopy Sciences – 3 ml/min (28°C)] at room temperature and allowed to recover for ≥1 h. One slice was then transferred into a 500 μl capacity recording chamber and perfused (submerged) at a flow rate of 2–3 ml/min (28–31°C) with ACSF that was bubbled with 95% O2-5% CO2.

“Blind patch” recordings were done in either the conventional whole cell mode or the nystatin-perforated patch technique using glass electrodes with tip resistance of 4.0–8.0 MΩ. In the perforated patch mode, series/access resistance of 10–30 MΩ was routinely attained in 2–20 min after the formation of a gigaohm (1–10 GΩ) seal. The internal recording solution used contained (in mM) 120 K-acetate, 5 MgCl2, 10 EGTA, and 40 HEPES. Nystatin was dissolved in dimethyl sulfoxide (DMSO) with Phuronic F127 and added to the internal solution to yield a final concentration of 450 μg/ml. The pH of the final solution was adjusted to 7.3. For recording in whole cell mode, the composition of the internal solution was (in mM) 135 K-glucconate, 8 NaCl, 0.2 EGTA, 10 HEPES, 2 Mg-ATP, and 0.2 GTP; pH was adjusted to 7.3 and osmolality of 270–280 mOsm. Bipolar tungsten stimulating electrodes were placed at the prefrontal cortex-accumbens border to evoke synaptic responses. Records were made using Axopatch 1D amplifiers in either voltage- or current-clamp modes. Reported resting potential for each cell was corrected for liquid junction potential by estimating the offset at the end of each experiment and adding it to the potential that was determined immediately after acquiring the cell.

Cells were voltage clamped at −80 mV, and input (Rinput) and access resistance (Ra) of all cells were monitored regularly throughout each experiment by applying a 20 mV hyperpolarizing pulse for 75–100 ms. Rinput was calculated from the steady-state current obtained during the pulse. The decay rate (τ) of the capacitance transient was taken as a measure of Ra. Cells obtained by either recording technique had similar Rinput and Ra (10–30 MΩ). Data from cells that showed >15% change in Ra were excluded from further analysis.

Evoked non-NMDA receptor-mediated pure EPSCs were isolated both pharmacologically, using 50 μM picrotoxin and biophysically by voltage clamping cells at −80 mV. Picrotoxin was present in the bath throughout each experiment. These evoked EPSCs were verified to be pure, non-NMDA receptor-mediated responses by their complete abolition in the presence of the non-NMDA receptor antagonist 6-7-dinitroquinoxaline-2,3-dione (DNQX; 5–10 μM). Except where indicated, all experiments in this report were conducted using these pharmacologically isolated evoked EPSCs. Pure NMDA receptor-mediated EPSCs were also isolated pharmacologically and biophysically by recording in the presence of picrotoxin (50 μM) and DNQX (10 μM) and holding the cells at depolarized potentials (approximately −55 mV). These responses were verified to be NMDA receptor-mediated by their complete abolition in the presence of 50–100 μM d-APV, a selective NMDA receptor antagonist.

All cells had a graded evoked synaptic response to increasing stimulation intensity (ranging from 0.4 to 50 V), and an intensity giving 50–60% of the maximum evoked EPSC was used to evoke test responses. In paired-pulse experiments, synaptic responses were triggered by the application of two consecutive stimuli separated by 50 ms. The ratio between the second response (P2) and the first response (P1) was calculated and used as the paired pulse ratio (PPR). All data were acquired using pClamp Software (Clampex 7 or 8, Axon Instruments) at sampling rates of 2–10 KHz and filtered at 500–1000 Hz, digitized at 10 KHz, and stored for off-line analysis. Hard copy chart records were also captured on HP and Gould chart recorders.

Excitatory postsynaptic current and potential amplitudes were measured from baseline to peak and taken as the synaptic strength at the chosen stimulus intensity. Responses were normalized by taking the mean of the last three to four responses prior to drug application and dividing the rest of the responses by this mean. These normalized values were then used for average plots. For these plots, all cells receiving the same treatment were aligned at the first minute of drug application and averaged over the entire period. All values are stated as mean ± SE. One-way ANOVA and post hoc tests, as indicated in RESULTS, were used to compare different values or treatments (SigmaStat). Significance was taken at the level of P ≤ 0.05. Graphing was done using SigmaPlot and CorelDraw software.

All drugs were bath-perfused at final concentrations indicated by dissolving aliquots of stock in the ACSF. SCH23390 and sulpiride were prepared daily and used within 24 h. Dopamine was prepared shortly before each bath application and used immediately. This prevented the need for antioxidant protection. Most drugs [including d-2-amino-5-phosphonovaleric acid (d-APV)] and routine laboratory chemicals were from Sigma except for 6-7-dinitroquinoxaline-2,3-dione (DNQX), haloperidol, sulpiride, SCH23390, dopamine, and 8-cyclopentyl-1,3-dimethylxanthine [8-cyclopentyl theophylline (8-CPT)], which were obtained from RBI. Substance P, [Sar9, Met5] SP (O2)11-SP, and L732 138, were from Tocris, and Phuronic F127 was from BASF-Germany.

RESULTS

The results reported in this study were obtained from recordings in 74 NAc cells using either “blind” conventional whole cell or nystatin-patch recording techniques. Cells recorded using either of these techniques showed no significant differences in both passive and active membrane properties. All these cells had resting membrane potentials of between −69 and −92 mV and resting input resistance of 105–635 MΩ.
(196 ± 30 MΩ), parameters that are similar to those previously reported for these cells using whole cell recording only (Kombian and Malenka 1994). All cells were voltage clamped at −80 mV, and pure EPSCs were isolated by blocking GABA_A receptor-mediated inhibition with 50 μM picrotoxin. The remaining inward synaptic current was then completely abolished by 5 μM DNXQ, indicating they were pure non-NMDA receptor-mediated EPSCs (n = 4). NMDA receptor-mediated responses, recorded in current-clamp mode only (EPSPs), were abolished by 50–100 μM p-APV (n = 5), indicating they were pure NMDA receptor-mediated EPSPs.

Substance P depresses evoked excitatory synaptic responses by activating NK1 receptors

Bath application of SP for 5–6 min caused a decrease in the amplitude of evoked non-NMDA receptor-mediated EPSCs in 27 of 30 cells tested (90%). The onset of action was between 2 and 3 min with a peak effect in about 5–6 min. One of the remaining cells had no response to SP while the other two responded with small increases in EPSC amplitude and action potential firing. The latter two cells may represent the aspiny cholinergic interneurons present in this nucleus. They are known to possess NK1 receptors on their somatodendrites (Murtra et al. 2000; Pickel et al. 2000) that can be excited by SP to produce these effects.

The SP-induced synaptic depression in the majority of NAc neurons was concentration-dependent, with maximum synaptic depression observed with a SP concentration of 1 μM (41.5 ± 3.6%, n = 6, Fig. 1). Above this concentration, the synaptic depressant effect tended to decline (Fig. 1B). The synaptic depressant effect, even at lower concentrations, only showed partial recovery after 8–15 min washout of SP (68 ± 9.3%, n = 5; Fig. 1, A and C). Similar to its effect on the above response, SP (1 μM) also depressed the amplitude of the non-NMDA receptor-mediated EPSP recorded in these neurons (Fig. 2). The magnitude of this depression by 1 μM SP was 53.5 ± 2.6% (n = 5), which was significantly higher than the depression of the non-NMDA receptor-mediated response (P < 0.05, unpaired t-test). As these cells rest at relatively negative potentials and basal excitatory synaptic transmission is mediated mainly by non-NMDA receptors, the rest of this study was done on the actions of SP on the non-NMDA receptor-mediated response.

To determine the type of neurokinin receptors mediating the SP effect, we used a selective agonist and antagonist. Bath application of the NK1 receptor selective agonist, [Sar⁹, Met(O₂)¹¹]-SP (King et al. 1997; Tousignant et al. 1990), at an equivalent concentration of 1 μM, also caused synaptic depression (42.7 ± 2.5%, n = 4; Fig. 3, A and C) with partial recovery on washout (65.1 ± 9.5%). This level of depression is similar to that produced by the endogenous peptide SP (P > 0.05, unpaired t-test), indicating that the agonist is equipotent with the endogenous transmitter in the depression of excitatory transmission. In the presence of a selective NK1 receptor antagonist, L732 138 (10 μM; MacLeod et al. 1994), SP (1 μM) no longer decreased the evoked EPSC amplitude (2.8 ± 5.1%, P > 0.05 compared with control, n = 4, paired t-test; Fig. 3, B and C). Taken together, these data indicate that SP depressed evoked EPSC in this nucleus by activating NK1 receptors.

Substance P depresses evoked EPSC amplitude by a presynaptic mechanism

The above results clearly indicate that SP and its agonist depress evoked pure EPSCs recorded in the majority of cells of the NAc. We next sought to identify the site within the synapse where SP acted to depress evoked EPSC amplitude. The locus of action of drugs to alter synaptic transmission has routinely been determined by a battery of tests used to distinguish presynaptic from postsynaptic actions. We applied several of these tests to establish the locus of action of SP. First, neither SP nor the NK1 receptor agonist, [Sar⁹, Met(O₂)¹¹]-SP, caused a change in the holding current in the majority (>95%) of the recorded cells, indicating they did not induce or block a resting conductance in these cells, although the synaptic response was depressed (Fig. 4). Furthermore, the application of a voltage pulse to monitor the input resistance (R_input) and access/series resistance in control conditions and in the presence of SP (1 or 2 μM) showed no difference in the R_input (283.4 ± 59 MΩ in control vs. 267 ± 51 MΩ, P > 0.05, n = 8; paired t-test, Fig.
FIG. 2. Substance P depresses evoked, NMDA receptor-mediated EPSPs in the nucleus accumbens. Aa: representative excitatory postsynaptic potential (EPSP) in a nucleus accumbens cell evoked at resting membrane potential. Ab: application of the non-NMDA receptor antagonist DNQX 10 μM abolished the response in Aa. Ac: when this cell was depolarized by about 20 mV and the stimulus strength slightly increased, a bigger EPSP that had a slower rise time and decay rate was elicited. Right panel shows the response in Aa scaled to that in Ac and superimposed. Bottom panel: sample traces of evoked NMDA receptor-mediated EPSPs showing depression by 1 μM SP (middle panel). Application d-APV(100 μM), an NMDA receptor antagonist, eliminated the remaining response (right panel). B: average time-effect plot (n = 5 cells) showing the effect of SP 1 μM and d-APV 100 μM on the NMDA receptor-mediated EPSP. In this and in C, at the peak of the effect of DNQX effect, a new baseline was re-established for the NMDA response and subsequent responses were normalized to this new baseline. C: bar graph summarizing the effects of the various drugs tested on the EPSPs. Asterisk indicates statistical significance compared with control and antagonists at P < 0.05. In this and in subsequent bar graphs, n values represent number cells tested.

4A) and no apparent change in access to the cell. Next, to see whether SP caused changes in the conductance of these cells in a voltage range outside of the resting membrane potential that might affect the evoked response, we applied slow voltage ramps (from −120 to −40 mV; at a rate of 4.5 mV/s) to the membrane and recorded the corresponding steady-state currents to yield current-voltage (I-V) curves. The curves generated at the peak of the SP synaptic depressant effect were superimposable on those obtained in control over the entire voltage range tested (Fig. 4A, n = 4). Finally we examined the effect of SP on the kinetics of the evoked EPSC. The decay constant (τ) of the evoked EPSC in control and at the peak of the SP-induced synaptic depression were compared. In six cells, τ in control was 11.2 ± 1.8 ms versus 10.0 ± 1.0 ms in the presence of 1 μM SP (P > 0.05, paired t-test; n = 6, Fig. 4B). Figure 4B shows that when the EPSC in the presence of 1 μM SP is scaled to the size of the control EPSC, their rise time and decay rate are the same. All these postsynaptic manipulations consistently showed that SP depressed the evoked potential without affecting postsynaptic characteristics of the recorded cells.

We next applied the paired pulse test, one that has frequently been used to indicate presynaptic actions of drugs (Kombian et al. 1996, 1997; Nicola et al. 1996; Zucker 1989; but see Kim and Alger 2001). When two successive synaptic stimuli were applied at a 50-ms interval, there was an increase in the amplitude of the second EPSC (P2) compared with the first EPSC (P1), thus yielding a paired pulse ratio (PPR; P2/P1) of greater than one (paired pulse facilitation; Fig. 5A). In the presence of 1 μM SP, both responses were depressed, but the first EPSC was more depressed than the second one, resulting in an enhancement in the PPR (11.2 ± 1.7% over control; P < 0.05, n = 5, Fig. 5). In the presence of [Sar⁹, Met (O₂)¹¹]-SP (1 μM), the NK1 receptor agonist used in this study, the enhancement was 29.4 ± 11% (P < 0.05, paired t-test, n = 4; Fig. 5C). SP-induced enhancement in PPR was blocked by L732 138 (10 μM), the NK1 receptor antagonist that had blocked the synaptic depression (−1.8 ± 8.3%, n = 4, P > 0.05 compared with control PPR, unpaired t-test, Fig. 5C). Also, PPR enhancement caused by [Sar⁹, Met (O₂)¹¹]-SP (1 μM) was blocked by L732 138 pretreatment. The enhancement in PPR caused by SP and the NK1 agonist, coupled with the
in the same cell, application of a ramp protocol (A2 scales for the EPSC and the postsynaptic membrane response are different). Pulse remained the same in control and in the presence of SP (note that the depressed the EPSCs amplitude, the membrane response to a square voltage/H9262 pure EPSCs recorded in control and in the presence of 1 around ‘H9270 depression caused by SP was mediated by DA, we blocked all. a H9262 effects in both curves aposed on the control trace (a), and the of the control trace (a) 732 S. B. KOMBIAN, K.V.V. ANANTHALAKSHMI, S. S. PARVATHY, AND W. C. MATOWE DE circuit H9262 a block the synapse. In this typical cell, the application of a pair of synaptic stimuli separated by 50 ms frequently resulted (SCH23390) and D2-like (sulpiride) receptor antagonists. Bath application of a combination of SCH23390 (30 μM) and sulpiride (10 μM) prevented the SP-induced synaptic depression (8.5 ± 5.5, P > 0.05 compared with control synaptic response, paired t-test, n = 4; Fig. 6). In another two cells, this combination also blocked the synaptic depressant effect of the SP receptor agonist, [Sar9, Met (O2)11]-SP. To determine if the SP-induced synaptic depression was mediated by DA D2-like receptors, we eliminated SCH23390, the DA D1-like receptor antagonist from the cocktail leaving sulpiride, the D2-like antagonist. Application of 1 μM SP in the presence of a blocking concentration of sulpiride (10 μM) still caused a depression of the evoked EPSC (38.7 ± 5.7, n = 3; Fig. 6B), an effect that was comparable to the synaptic depression under control conditions (41.5 ± 3.6%, n = 6, P > 0.05; unpaired t-test). This suggests that the blockade by the cocktail was due to SCH23390 as previously reported (Harvey and Lacey 1996; Nicola et al. 1996; Pennartz et al. 1992). This was confirmed in an additional four cells that were pretreated with SCH23390 (30 μM) alone. Application of SP (1 μM) in the presence of this blocking concentration of SCH23390 did not produce a lack of effect on postsynaptic characteristics of the recorded cells, are consistent with the hypothesis that SP produces its synaptic depressant effect in this nucleus by a presynaptic mechanism.

Dopamine receptor antagonists block substance P-induced synaptic depression

Neurochemical studies indicate that SP increases the levels of extracellular dopamine in the NAc (Boix et al. 1992; Cadoret al. 1986; Krasnova et al. 2000) and DA has been shown to decrease EPSCs recorded in these cells (Harvey and Lacey 1996; Nicola and Malenka 1998; Nicola et al. 1996). We explored to see whether the synaptic depression induced by SP was through the enhancement of extracellular DA, which then acted on its receptors to depress the evoked EPSC amplitude. Although, it has previously been reported that DA and the psychostimulants cocaine and amphetamine (indirectly through DA) depress evoked EPSC in the NAc by activating presynaptic D1-like receptors (Harvey and Lacey 1996; Nicola et al. 1996), other studies (e.g., in the neostriatum) reported that DA depressed excitatory synaptic transmission by activating D2 receptors (Hsu et al. 1995; Levine et al. 1996; Umemiy and Raymond 1997). To test the hypothesis that the synaptic depression caused by SP was mediated by DA, we blocked all DA receptors using a cocktail that contained both D1-like (SCH23390) and D2-like (sulpiride) receptor antagonists. Bath application of a combination of SCH23390 (30 μM) and sulpiride (10 μM) prevented the SP-induced synaptic depression (8.5 ± 5.5, P > 0.05 compared with control synaptic response, paired t-test, n = 4; Fig. 6). In another two cells, this combination also blocked the synaptic depressant effect of the SP receptor agonist, [Sar9, Met (O2)11]-SP. To determine if the SP-induced synaptic depression was mediated by DA D2-like receptors, we eliminated SCH23390, the DA D1-like receptor antagonist from the cocktail leaving sulpiride, the D2-like antagonist. Application of 1 μM SP in the presence of a blocking concentration of sulpiride (10 μM) still caused a depression of the evoked EPSC (38.7 ± 5.7, n = 3; Fig. 6B), an effect that was comparable to the synaptic depression under control conditions (41.5 ± 3.6%, n = 6, P > 0.05; unpaired t-test). This suggests that the blockade by the cocktail was due to SCH23390 as previously reported (Harvey and Lacey 1996; Nicola et al. 1996; Pennartz et al. 1992). This was confirmed in an additional four cells that were pretreated with SCH23390 (30 μM) alone. Application of SP (1 μM) in the presence of this blocking concentration of SCH23390 did not produce a
significant decrease in the evoked EPSC (1.2 ± 4.5 P > 0.05, compared with control, paired t-test). Finally, haloperidol (50 μM), a relatively selective D2-like receptor antagonist, but which may also have D1-like activity at high concentrations (K1 for D1/D5 approximately 100 nM; Seeman and Van Tol 1994), also blocked the SP-induced synaptic depression (Fig. 6B). Taken together, these results suggest that SP acts to increase the extracellular level of DA, which then acts on D1-like receptors to cause a decrease in evoked EPSC amplitude.

**Substance P-induced synaptic depression is blocked by an adenosine A1 receptor antagonist but not by an NMDA receptor antagonist**

The observed effect of SP to decrease excitatory synaptic transmission in the NAc and its blockade by dopamine receptor antagonists clearly indicates it acts indirectly through the release of dopamine. Harvey and Lacey (1997), however, reported that the synaptic depressant effect of dopamine itself was also indirect through an inhibitory feedback action of adenosine released from the postsynaptic neuron as a result of facilitation of NMDA receptor action by dopamine, acting on D1-like receptors. To test whether a similar feedback mechanism was operating in this SP-induced EPSC depression, we attempted to block SP effects using the adenosine A1 receptor-selective antagonist 8-CPT and the NMDA receptor antagonist APV.

Bath application of 1 μM 8-CPT caused a rapid increase in evoked EPSC amplitude by 73.1 ± 17.5% (n = 10, Fig. 7). Subsequent application of 1 μM SP in the presence of 8-CPT resulted in a synaptic depression of 1.6 ± 1.5% (n = 6) compared with the synaptic depression of 41.5 ± 3.6% in control (P < 0.05; unpaired t-test, Fig. 7B). In two of these cells, when 8-CPT was washed out, SP alone subsequently depressed the evoked EPSC amplitude by an average of 28.3%.

In addition to blocking the SP-induced synaptic depression, 8-CPT (1 μM) also blocked synaptic depression caused by DA (50 μM) in these cells (40.9 ± 13.5% in control vs. 0.5 ± 4.6% in the presence of 1 μM 8-CPT, P < 0.05; paired t-test; n = 3). In contrast to the ability of 8-CPT to block the SP-induced synaptic depression, APV at 100 μM, a concentration that was previously shown to abolish NMDA receptor-mediated EPSPs (see Fig. 2), did not block the SP-induced synaptic depression (35.8 ± 8.4%, n = 5; P > 0.05 compared with control SP effect; unpaired t-test; Fig. 7B). Furthermore, APV at the same concentration failed to block DA-induced synaptic depression (n = 2 cells, data not shown). These results indicate that SP depresses evoked EPSC amplitude in the NAc by employing adenosine that may be produced without the involvement of NMDA receptor activation (Harvey and Lacey 1997; Nicola and Malenka 1997).

**DISCUSSION**

Our results show that SP, a neuropeptide that is present in the NAc, depresses excitatory synaptic transmission in this nucleus indirectly by increasing the extracellular levels of DA. Dopamine then acts on appropriate DA receptors to increase extracellular levels of adenosine. Adenosine then acts on pre-synaptic A1 receptors located on glutamatergic terminals to decrease glutamate release and reduce evoked EPSCs.

Although there are three main types of neurons in the NAc, the predominant medium spiny GABAergic projection neurons and the fewer GABAergic and cholinergic interneurons (Meredith 1999; O’Donnell and Grace 1993; Pennartz and Kitai 1991; Pennartz et al. 1994), it was not possible to distinguish between these two cell populations in this study. However, due to their relative abundance (>90%), most of the cells recorded in this study likely represent medium spiny...
GABAergic neurons. Exogenously applied SP, an excitatory peptide (Collingridge and Davies 1982; Davies and Dray 1976; Otsuka and Konishi 1976) paradoxically produced a concentration-dependent depression of evoked EPSC. This effect was biphasic, with a peak effect occurring at 1 μM but decreased at higher concentrations. Similar biphasic dose-response curves have been reported for SP in vivo in studies where both neurochemical and behavioral parameters were monitored (Kalivas and Miller 1984b). The exact mechanism by which this happens is not known but could involve either SP receptor desensitization at high concentrations or the activation of additional neurokinin receptors whose effects are opposite to those of NK1 receptors. Since repeated injections of SP at low concentrations have produced consistent behavioral responses in rats (West and Michael 1991), desensitization does not appear to occur at low concentrations such as those used in this study. The SP synaptic depressant effect in vitro showed only partial recovery within the time frame of our experiments. This partial recovery may be responsible for the prolonged behavioral effects of SP in vivo (Boix et al. 1994).

The mimicry of the SP synaptic effect by the NK1 receptor agonist (Tousignant et al. 1990) and its blockade by a NK1 receptor selective antagonist (Macleod et al. 1993) are both consistent with immunohistochemical studies showing the presence of NK1 receptors in both somatodendrites and terminals in the NAc (Murtra et al. 2000; Pickel et al. 2000). Thus SP produces this effect by activating SP-preferring receptors, the NK1 receptors.

All passive membrane processes assessed in this study including resting conductances/resting potential, input resistance, and non-NMDA receptor response kinetics were not altered by the presence of SP in most cells tested. The lack of direct postsynaptic effects of SP and its receptor agonist suggests that NK1 receptors may be lacking or very sparse on the recorded neurons. This lack of postsynaptic effect is consistent with immunocytochemical and electron microscopic data that indicate that NK1 receptors are abundant only on the perikarya and dendrites of the cholinergic interneurons, but rare on the predominant medium spiny GABAergic projection neurons (Pickel et al. 2000). Since most of the cells recorded in this study were likely medium spiny GABAergic neurons, it is not surprising therefore that no postsynaptic effects of SP were detected. In contrast to its lack of postsynaptic effects, SP increased PPF in these cells, changes often interpreted to indicate presynaptic mechanisms (Kombian et al. 1996, 1997; Manabe et al. 1993; Zucker 1989). This is consistent with previous reports by Mitrovic and Napier (1998), who, using in vivo micro-iontophoresis of SP, concluded that SP acted presynaptically to decrease amygdala-stimulated evoked excitation in the NAc.

Most reported actions of SP indicate it is an excitatory peptide (Nakajima et al. 1991a; Otsuka and Konishi 1976; Stanfield et al. 1985), which may act to increase the firing rate of neurons in the substantia nigra (Collingridge and Davies 1982; Davies and Dray 1976), the VTA (West and Michael 1991), and primary sensory neurons (Otsuka and Konishi 1976). It was thus surprising that SP depressed evoked EPSCs in the NAc. To accomplish this inhibition, SP would have to recruit other neuromodulators to mediate this effect. Several lines of evidence suggest that this indeed is the case. Neurochemical studies indicate that SP increases the extracellular level of DA (Boix et al. 1992b; Cador et al. 1989; Elliott et al. 1986b; Kalivas 1985a), while behavioral studies indicate that its actions not only resemble those of DA (Deutch et al. 1985; Eison et al. 1982a; Kalivas and Miller 1984b; West and Michael 1991) but are actually dependent on DA, because these effects can be blocked by chemical lesioning of DA terminals in the NAc (Stinus et al. 1978), 2) be prevented by administration of DA receptor antagonists (Kelley et al. 1979), and 3) are augmented by DA or DA releasing chemicals such as amphetamines (Eison et al. 1982b; Kalivas and Miller 1984b; West and Michael 1991).

In addition to the neurochemical and behavioral convergence of SP and DA effects, light and electron microscopic studies also reveal that most of the SP-like immunoreactive terminals in the NAc are frequently associated with dopaminergic terminals (Huston and Holzhauer 1988; Ljungdahl et al. 1978; Steffensen et al. 1998). The cytoarchitectural arrangement of these terminals therefore permits an interaction between them. Finally, the SP-prefering receptors, the NK1 receptors, are located both on the somatodendrites of the dopaminergic cell bodies located in the VTA and the axon terminals in the NAc (Mantyh et al. 1984). Activation of these receptors in the VTA excites these cells to fire action potentials resulting in DA release in their terminal fields including the NAc (West and Michael 1991). In addition, activation of these same receptors on DA terminals in the NAc also modulates intra-accumbens DA levels and produces behavioral effects similar to those of intra-VTA injections (Kalivas and Miller 1984b). Based on these facts and our current results, we suggest that SP produced the observed depression of excitation indirectly by causing an increase in DA levels. This could be achieved by either blocking the breakdown of released DA or by causing its release from terminals, or both. While the evidence for its effects on the metabolism of DA are conflicting (Cador et al. 1989; Elliott et al. 1986a), available evidence suggests that it is most likely the latter since SP has been shown to release DA from synaptosomes prepared from NAc (DeBelleruche and Gardiner 1983). The complete blockade of the SP effect by DA receptor antagonists confirmed that SP depressed excitatory synaptic transmission in the NAc by first increasing the turnover of DA, which then acts on DA receptors to depress the amplitude of the evoked EPSC. The receptors involved in this case are likely the D1-like receptors as both DA and SP effects were blocked by SCH23390, and these receptors have previously been reported to mediate DA-induced synaptic depression in this nucleus (Harvey and Lacey 1996; Nicola et al. 1996; but see O’Donnell and Grace 1994).

Another possible indirect mechanism of action may be one recently described by Harvey and Lacey (1997), whereby a synergistic interaction between DA, acting on D1 receptors and glutamate acting on NMDA receptors, leads to an increase in the release of adenosine, a neuromodulator that depresses excitatory transmission in this nucleus (Uchimura and North 1991). The blockade of SP-induced synaptic depression as well as DA-induced synaptic depression by 8-CPT, an adenosine A1 receptor antagonist, supports the utilization of adenosine as the most likely direct mediator of excitatory synaptic depression in the NAc. This effect, while in agreement with the report by Harvey and Lacey (1997), contradicts the findings reported by Nicola and Malenka (1997), which did not reveal a requirement for adenosine in mediating DA-induced synaptic depres-
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Sion in this nucleus. Regarding the role of NMDA receptors, we did not observe a requirement for NMDA receptor activation in the SP-induced synaptic depression. This finding, while in agreement with that of Nicola and Malenka (1997), contradicts the findings by Harvey and Lacey (1997). Whether SP plays a direct role, alone or in concert with DA, in the generation of adenosine is not yet known. The controversy on the mechanism of EPSC depression by DA and its indirect agonists in the NAc remains to be resolved. This may require using multiple techniques such as neurochemistry coupled with electrophysiological recordings in naive and DA-lesioned animals. Such studies would reveal whether DA directly affects glutamate release or adenosine production and the role of other receptors such as NMDA and NK1 receptors.

Taken together, our evidence suggests that SP, an excitatory neuropeptide, acts locally to excite meso-accumbens DA terminals in the NAc leading to an efflux of DA into the extra-cellular space. It may accomplish this by modulating inwardly rectifying potassium channels that SP has been reported to inhibit (Nakajima et al. 1991b, 1993). DA then acts on DA D1-like receptors to generate adenosine. This action of DA does not appear to require the action of NMDA receptor activation as reported by Harvey and Lacey (1997). The latter may then act as the final mediator in this cascade by activating A1 receptors located on presynaptic glutamatergic terminals to depress excitatory synaptic transmission (Fig. 8; but see Nicola and Malenka 1997).

The predominant projection neurons of this nucleus, the medium spiny GABAergic neurons, generally rest at relatively negative potentials and are thus strongly dependent on afferent excitation to generate their output. The depressant effect of SP on afferent excitation would be predicted to limit the ability of these cells to reach AP threshold and hence moderate the generation of their output. As SP in the NAc is from these same projection cells, its release would serve as a negative feedback control to curtail excessive afferent excitation. As well, since these cells are known to form extensive axon collateral networks within the NAc, the firing of a group of neurons would likely result in the suppression of firing of neighboring neurons that receive collateral innervation from this group of activated cells. SP’s action may therefore serve to select and sharply focus NAc’s output, thus filtering out competing or unnecessary cortical inputs.

As a consequence of the complex synaptic organization within the NAc whereby several inhibitory and excitatory neurotransmitters/modulators converge onto the same nucleus or neurons (Fig. 8), coupled with a complex chain of synaptic connections (using both inhibitory and excitatory transmitters) that tightly regulate the final behavioral output, numerous possibilities exist for synaptic excitation or inhibition at any level in this chain to translate into the same behavioral output. The behavioral consequences of administration of SP to animals, such as increased locomotor activity, are similar to those produced by psychostimulants cocaine and amphetamine, which are mediated by DA in the NAc (Kuhr et al. 1991; Ritz et al. 1987). Our demonstration here that SP, an endogenous neuropeptide produces synaptic effects similar to those produced by these psychostimulants (Nicola et al. 1996) suggests that SP may serve as an endogenous psychogenic peptide. Since SP acts neurochemically, behaviorally, and now at the cellular and synaptic levels like the exogenous psychoactive substances, it may be involved in the physiology and/or pathophysiology of reward and addictive behaviors. As well, SP may play a role in the pathogenesis of psychiatric disorders where the NAc is known to play a major role and the main disturbance is in DA and its receptors.

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