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Neurokinin Receptors Differentially Mediate Endogenous Acetylcholine Release Evoked by Tachykinins in the Neostriatum

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The regulation of neostriatal cholinergic function by tachykinins (TKs) has been studied by measuring endogenous ACh released from rat neostriatal slices. Septide (SEP; a highly selective substance P analog), neurokinin A (NKA), and neurokinin B (NKB) elicited endogenous ACh release in a concentration-dependent manner. The rank order in potency was the following: NKB ($EC_{50} \approx 0.5$ nM) > NKA ($EC_{50} \approx 7$ nM) > SEP ($EC_{50} \approx 12$ nM). Spantide (SPA) was less effective (39% inhibition) than [D-Arg^6, D-Trp^{7,9}, *N*-Methyl-Phe^8]-substance P fragment 6-11 (53% inhibition) at antagonizing ACh release evoked by SEP and NKA. Smaller doses of the antagonists inhibited the effects of SEP compared to NKA, and the effects of NKB could only be antagonized by SPA. These findings suggest the involvement of the three neurokinin (NK) receptors in ACh release evoked by TKs with the following rank order: $NK_3 > NK_2 > NK_1$.

6-Hydroxydopamine lesions of nigrostriatal neurons and tetrodotoxin (TTX) intoxication of striatal tissue revealed two different patterns of regulation of cholinergic function by TKs. On the one hand, SEP and NKA evoked ACh release, independently of the nigrostriatal dopaminergic system, by acting on NK_1 and NK_2 receptors that are probably localized on the somatodendritic field of cholinergic neurons receiving substance P terminals. On the other hand, dopaminergic terminals seem to regulate NKB neurons that modulate cholinergic neurons, because NKB-evoked ACh release decreased by 24% in the denervated striata. In addition, TTX partially blocked (50%) ACh release evoked by NKB, suggesting that NKB acts on NK_3 receptors at both the nerve terminals and the somatodendritic field of cholinergic neurons. Therefore, NKB neurons could play a crucial role in regulating cholinergic terminals by partially mediating the dopaminergic influence on cholinergic neurons. This finding may provide some clues in the organization of the basal ganglia and in the understanding of basal ganglia disorders.

The neostriatum is known to contain some of the highest amounts of cholinergic markers in the CNS (Kása, 1986). Cholinergic neurons in the rat neostriatum have been identified by ChAT immunocytochemistry as large or giant aspiny interneurons (Bolam et al., 1984; Wainer et al., 1984; Phelps et al., 1985). Although cholinergic neurons represent a small population in the neostriatum (McGeer et al., 1984; Graybiel et al., 1986), their widespread dendritic and axonal fields, high sensitivity to small depolarizing potentials, and tonic firing place them in an excellent position to act as modulators of the excitability of neostriatal projection neurons in advance of the onset of movement-related neostriatal activity (Wilson et al., 1990). Thus, all these properties endow cholinergic neurons with a key role in basal ganglia functions and disorders, resulting in a very sensitive marker of neostriatal function.

Tachykinins (TKs) constitute a neuropeptide family widely distributed and active in both the CNS and peripheral tissues. The endogenous mammalian members of this family are substance P (SP), neurokinin A (NKA), and neurokinin B (NKB; Maggio, 1988). Although the immunohistochemical localization of these peptides has been difficult because of the cross-reactivity between antibodies to different TKs, two distinct populations of medium-sized neurons containing SP immunoreactivity have been described in the neostriatum (Bolam et al., 1983; Bolam and Izzo, 1988; Gerfen and Young, 1988). Furthermore, SP-containing terminals have been reported to synapse on the somatodendritic tree of cholinergic neurons (Bolam et al., 1986) and on medium-sized neurons (Bolam et al., 1983; Bolam and Izzo, 1988). Recently, *in situ* hybridization studies have permitted the differentiation between SP and NKB transcripts (Warden and Young, 1988). In the neostriatum, cells expressing NKB have been found to be present either scattered or in small clusters projecting to the globus pallidus (Burgunder and Young, 1989). Furthermore, these cells coexpress neurotransmitters present in two medium-sized neurons: SP is found in 59% and enkephalin in 25% of the NKB-positive cells (Burgunder and Young, 1989). At the moment, specific probes for the histochemical localization of NKA are not available. However, lesion studies have suggested that SP and NKA could be coexisting in some of the striatonigral-projecting neurons (J. M. Lee et al., 1986; Lindefors et al., 1986), which also give extensive striatal innervation (DiFiglia et al., 1980; Wilson and Groves, 1980; Somogyi et al., 1981). Molecular biology studies have shown that both SP and NKA are generated from β- and γ-preprotachykinin A mRNAs (Krause et al., 1987), suggesting that the source of SP/NKA terminals in the neostriatum is common in some neurons.

The endogenous TKs (SP, NKA, and NKB) act as preferred, but not exclusive, endogenous ligands of the three classes of neurokinin (NK) receptor subtypes, NK_1, NK_2, and NK_3, respectively (Quirion and Dam, 1988; Watson and Abbot, 1990). The activation of NK receptors induces excitatory effects in

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several neural systems and releases many neuroactive substances (Maggio, 1988; Kangrga and Randic, 1990; Reid et al., 1990). Both *in vitro* (Lindefors et al., 1985) and *in vivo* (Lindefors et al., 1989) experiments have shown that TKs are released in a Ca^{2+}-dependent fashion in the neostriatum. Furthermore, autoradiographic studies have revealed moderate levels of the three classes of NK receptors in this nucleus (Saffroy et al., 1988; Dam et al., 1990). These data, together with the subcellular localization of TKs in synaptosomes and vesicle fractions (Diez-Guerra et al., 1987), are consistent with the role of TKs as neurotransmitters in the neostriatum. According to this role, SP has been reported to increase the firing rate of some striatal neurons (Le Gal La Salle and Ben-Ari, 1977) and to evoke the release of ^{3}H-dopamine (Starr, 1978, 1982; Petit and Glowinski, 1986; Baruch et al., 1988), endogenous dopamine (Reid et al., 1990), ^{3}H-5-HT (Starr, 1978), and ^{3}H-Met-enkephalin (Del Rio et al., 1983) in the neostriatum. Although NKA and NKB have also been reported to evoke the release of ^{3}H-dopamine (Petit and Glo winski, 1986; Glowinski et al., 1988), much less is known about the neurotransmitter role of NKA or NKB in the neostriatum, and evidence in favor of the regulation of striatal interneurons or intrastriatal circuits has not been obtained.

The present study was designed to investigate the regulation of neostriatal cholinergic neurons by TKs. Endogenous ACh release from neostriatal slices was continuously measured by means of a chemiluminescent method. The aims of this study were (1) to determine the role of activation and blockade of the three NK receptors on the regulation of endogenous ACh release, (2) to establish NK dependence on the dopaminergic system by means of 6-hydroxydopamine (6-OHDA) denervation, and (3) to depict the possible localization of the receptor(s) involved in such regulation by using the fast sodium channel blocker tetrodotoxin (TTX). The results indicate a differential pattern of tachykininergic regulation of ACh release. Our data provide first evidence of a novel excitatory regulation of neostriatal cholinergic interneurons by SP, NKA, and NKB.

Materials and Methods

Materials. ACh chloride, choline oxidase (EC 1.1.3.17), HRP type VI (EC 1.11.1.7), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), AChE type VI-S (EC 3.1.1.7), 6-hydroxydopamine hydrochloride, septide (SEP; [*p*-Glu^6, Pro^9]-substance P fragment 6-11), neurokinin A (also known as substance K, α-neurokinin, neuromedin L), neurokinin B (also known as β-neurokinin or neuromedin K), spantide (SPA; [D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P), and [D-Arg^6, D-Trp^{7,9}, *N*-methyl-Phe^8]-substance P fragment 6-11 (SPAF) were purchased from Sigma. Tetrodotoxin was supplied by Boehringer Mannheim.

6-Hydroxydopamine lesions. Male Sprague–Dawley rats (150–250 gm) were anesthetized with ketamine (150 mg/kg, i.p.) and placed in a David Kopf sterotaxic apparatus (DK 900). The incisor bar was placed 5 mm above the interaural line. A microinjection cannula was implanted into the medial forebrain bundle at the following coordinates: 1.6 mm caudal to bregma, 1.3 mm lateral to the midline, and 8.4 mm under the brain surface, according to the atlas of Pellegrino et al. (1979). Unilateral 6-OHDA (8 μg/2 μl) was injected at a rate of 1 μl/min, using a Harvard Apparatus syringe infusion pump 22. Rats were tested 1 month after surgery for contralateral circling behavior with apomorphine (0.5 mg/kg, s.c.) as described previously (Arenas et al., 1991). Only the animals that rotated contralateral to the lesion at a rate of at least 5 rpm during 1 hr were used in this study.

Tissue sampling. Lesioned and unlesioned rats were killed by decapitation. Brains were removed, and neostriata were quickly dissected out. Slices of about 4 mg wet weight and 500 μm thick were prepared. The slices were placed in a standard saline solution (NaCl, 136 mM; KCl, 5.6 mM; $MgCl_2$, 1.2 mM; Tris/HCl, 10 mM, pH 7.4; and glucose, 5 mM) at room temperature and equilibrated with a mixture of O_2 (95%) and CO_2 (5%) for 1 hr as previously described (Alberch et al., 1985, 1990; Arenas et al., 1990a).

Endogenous ACh assay. ACh release was measured by the chemiluminescent method described by Israël and Lesbats (1982) with some modifications (Arenas et al., 1990b). This method is based on the luminol–peroxidase chemiluminescent reaction, which uses the H_2O_2 generated by the oxidation of choline to betaine. In this assay, the slices were placed in a tube containing the luminescent mixture: NaCl, 136 mM; KCl, 5.6 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 5 mM; Tris/HCl, 10 mM, pH 7.7; choline oxidase, 2.5 IU/ml; HRP, 0.01 mg/ml; luminol, 5 μM; and AChE, 5 IU/ml. The tube was placed in front of the photomultiplier, and ACh release was evoked by adding KCl (50 mM) or TKs at different concentrations ranging from 10 pM to 10 μM. The peak of released ACh, recorded for each sample, was compared with the peak obtained by the injection of an ACh standard dose of 100 pmol in the same tube. Finally, the slices were weighed, and the results were expressed in nmol of ACh per gram of wet tissue per minute.

Incubations. Incubations with TTX were performed by immersing the slices for 12 min in a tube containing the standard saline solution plus 0.3 μM TTX. After this period, the slices were placed in another tube containing the luminescent mixture and subsequently depolarized with TKs or KCl (50 mM). Incubations with the antagonists were performed directly in the tube containing the chemiluminescent mixture. Spantide and [D-Arg^1, D-Trp^{7,9}, *N*-methyl-Phe^8]-substance P fragment 6-11 were injected 1 min before depolarization with the corresponding agonist.

All the drugs injected into the luminescent mixture were tested for their ability to modify the light emission. None of the peptides used in the experiments quenched or enhanced the chemiluminescent reaction itself.

Results

Effects of TK agonists on ACh release

The three TK agonists SEP, NKA, and NKB elicited ACh release from rat neostriatal slices in a concentration-dependent manner (Fig. 1). The relative efficacy of the TKs to evoke ACh release with respect to ACh release elicited by KCl (50 mM) was the following: NKA, 70–78%; NKB, 70–73%; and SEP, 60–63% (KCl control values, 9.01 ± 0.28 nmol ACh/gm wet tissue/min). Maximal release of ACh was obtained at the following concentrations: ≥0.01 μM NKB, ≥0.1 μM NKA, and ≥1 μM SEP. In the absence of Ca^{2+}, ACh release evoked by TKs was abolished (data not shown).

The relative potencies of the TKs on endogenous ACh release were determined from the concentration–response curves (Fig. 1). The rank order of the relative potency of the three agonists was the following: NKB > NKA > SEP, with an EC_{50} of approximately 0.5 nM for NKB, 7 nM for NKA, and 12 nM for SEP.

Effects of TK antagonists on ACh release evoked by TKs

To determine whether TK-evoked ACh release was specific, we used the TK antagonists SPA and SPAF. SPA at a concentration of 10 μM antagonized 35–39% of the excitatory effects of 1 μM SEP, 0.1 μM NKA, and 0.01 μM NKB (Fig. 2). The excitatory effects of 1 μM SEP and 0.1 μM NKA were inhibited by 52% with a concentration of SPAF of 10 μM (Fig. 3). However, at this same concentration of SPAF, the effects of NKB were not inhibited. Thus, ACh release elicited by the three TK agonists shows a differential sensitivity to blockade by SPA and SPAF.

Effect of TTX on striatal ACh release evoked by TKs

In order to study the dependence on fast Na^+ channels of the excitatory effects of TKs on ACh release, neostriatal tissue was incubated with TTX (0.3 μM) before KCl (50 mM) or TK depolarization. As we previously described (Arenas et al., 1990a), TTX significantly reduced, by about 30%, ACh release evoked

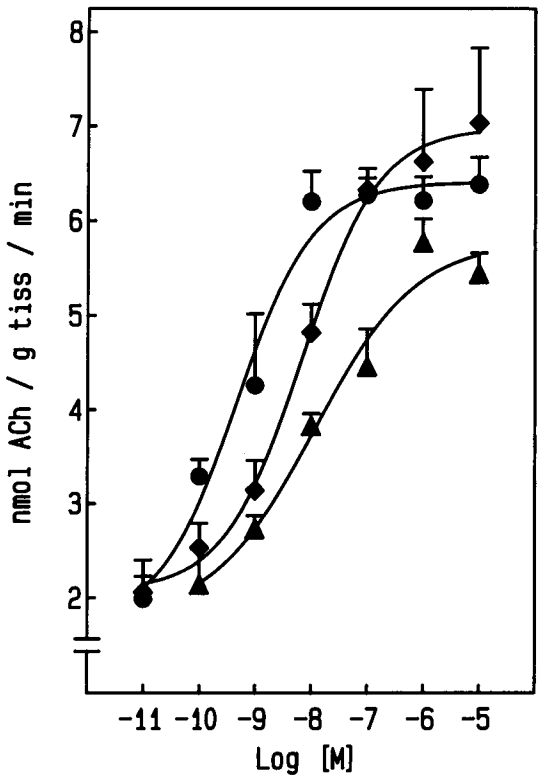

Figure 1. Dose–response curves of the excitatory effects of TKs on endogenous ACh release from rat neostriatal slices. NKB (*circles*), NKA (*diamonds*), or SEP (*triangles*) were directly injected at different concentrations into the detection medium containing the slice. Endogenous ACh release was immediately triggered and registered on line. Each *point* corresponds to the mean value ± SEM of three or more different experiments performed with duplicate or triplicate determinations.

by KCl (Fig. 4). TTX blocked by 70% the ACh release elicited by SEP (1 μM) or NKA (0.1 μM), similar to that described for glutamate (Arenas et al., 1990a). In contrast, TTX partially inhibited (by 50%) ACh release evoked by 0.01 μM NKB (Fig. 4).

Effects of unilateral injection of 6-OHDA in the nigrostriatal pathway on ACh release evoked by TKs

ACh release elicited by KCl or TKs from the nonlesioned neostriatum was not significantly different from ACh release in nonlesioned animals. Similarly, ACh release evoked by KCl (50 mM), SEP (1 μM), or NKA (0.1 μM) in the ipsilateral side to the 6-OHDA lesion was not different from that of the nonlesioned side. However, ACh release evoked by NKB (0.01 μM) in the ipsilateral side was reduced 24% with respect to the contralateral side (Fig. 5), suggesting a desensitization of the cholinergic response to NKB.

Discussion

Effects of TKs on striatal ACh release

In the present study, endogenous ACh release was elicited from rat neostriatal slices by SEP, a potent and selective NK_1 agonist (C.-M. Lee et al., 1986; Wormser et al., 1986; Laufer et al., 1988), and by NKA and NKB, the endogenous agonists of NK_2 and NK_3 receptors, respectively (Quirion and Dam, 1988; Watson and Abbot, 1990). It is interesting to note that our dose–response studies showed sigmoidal curves for the three agonists, suggesting the involvement of single saturable receptors for each response. This could be due either to the activation of NK_3 receptors by higher doses of NKA and SEP, or to the activation of NK_2 and NK_1 receptors by NKA and SEP, respectively. This latter possibility is in agreement with other studies (Baruch et al., 1988), which showed that the two endogenous agonists, NKA and NKB, had no cross-activity and clear differential effects on nigrostriatal dopaminergic neurons at very similar concentrations to those used in the present study. Furthermore, the application of NKA in the substantia nigra pars compacta has also been reported to be more effective than SP at exciting nigral dopaminergic neurons (Innis et al., 1985) and to require a 10-fold lower concentration than SP to increase the release of ^{3}H-dopamine in the caudate nucleus (Baruch et al., 1988). In addition, NKA has also been reported to be 10 times more potent than SP at increasing the locomotor activity in rats when infused into the ventral tegmental area (Kalivas et al., 1985). However, the most potent TK at evoking ACh release in the neostriatum was NKB, which, in contrast, has been reported to have no effect on ^{3}H-dopamine release in the caudate nucleus when applied in the substantia nigra pars compacta (Baruch et al., 1988). These data are in good agreement with the very low concentrations of NKB in the substantia nigra. Therefore, the presence of small populations of both NKB and cholinergic neurons in the neostriatum suggests that the potent effect of NKB on cholinergic neurons reflects a very close link between both NKB and cholinergic neurons.

Characterization of receptors mediating physiological effects has been largely based on the use of specific high-affinity antagonists, but for the TKs no such antagonists have been found. TK antagonists currently available are not fully specific and sometimes antagonize the actions of all TKs (Maggio, 1988). However, because NK receptor subtypes exhibit a differential sensitivity to blockage by various antagonists, these antagonists can provide information on the receptors involved. In order to determine the participation of different receptor subtypes, we used SPA, a relatively nonselective TK antagonist (C.-M. Lee et al., 1986; Iverfeldt et al., 1990), and SPAF, a relatively selective antagonist for the NK_1 receptor (Laufer et al., 1985). Because SPA antagonized the excitatory effects of SEP, NKA, and NKB on ACh release, it provided confirmation that ACh release evoked by TKs is due to the activation of NK receptors. Furthermore, the potency of SPA at antagonizing the effects of TKs on ACh release coincides with the affinity pattern of SPA for NK receptors ($NK_1 > NK_2 > NK_3$) in binding experiments (C.-M. Lee et al., 1986; Buck and Shatzer, 1988). Similarly, our results with SPAF are consistent with the pattern of receptors antagonized by SPAF: $NK_1 \geq NK_2$ (Laufer et al., 1985; Buck and Shatzer, 1988), with no effect on NK_3 receptors (Laufer et al., 1985). Thus, SPAF shares with SPA the relatively high affinity for NK_1 receptors and the 10-fold greater selectivity for NK_1 receptors with respect to NK_2 receptors, but differs from SPA in its inactivity on NK_3 receptors and its higher effectivity (52% inhibition by SPAF vs. 38% by SPA).

The present data, according to the characteristics of NK receptors (C.-M. Lee et al., 1986; Watson and Abbot, 1990), are consistent with a major involvement of NK_3 receptors in the excitatory influence of TKs on endogenous ACh release from rat neostriatal slices. Furthermore, our results with the antagonists suggest that the rank order in potency of the three agonists at evoking endogenous ACh release (NKB > NKA > SEP) could correspond to the activation of the three NK receptors coupled

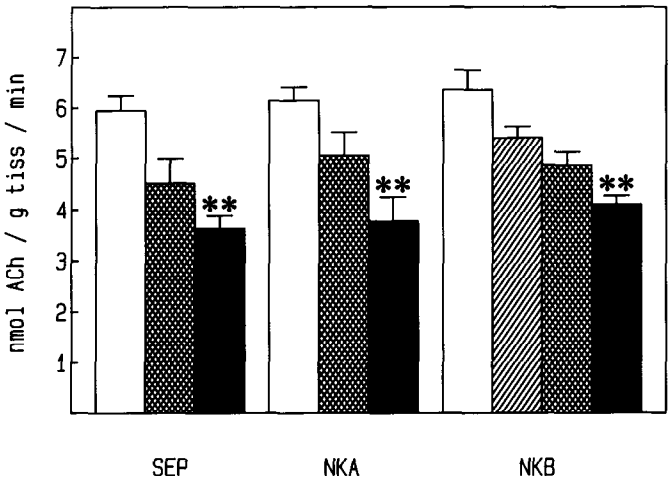

Figure 2. Effects of SPA on endogenous ACh release evoked by TKs. No antagonist (*open bars*) or different concentrations of SPA ([D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P), 0.1 μM (*hatched bar*), 1 μM (*cross-hatched bars*), or 10 μM (*solid bars*), were injected into the detection medium containing the slice, 1 min before depolarization with 1 μM *SEP*, 0.1 μM *NKA*, or 0.01 μM *NKB*. SPA required 10-fold higher concentrations than SEP, 100-fold higher concentrations than NKA, and 1000-fold higher concentrations than NKB (10 μM) to antagonize partially their effects on ACh release, suggesting the following rank order in potency: SEP > NKA > NKB. Each value corresponds to the mean ± SEM of four or more different experiments performed with duplicate or triplicate determinations. The statistical significance was evaluated by the Student's *t* test for nonpaired data with Bonferroni's correction for four comparisons. **, $P < 0.0025$ compared with the corresponding open bar.

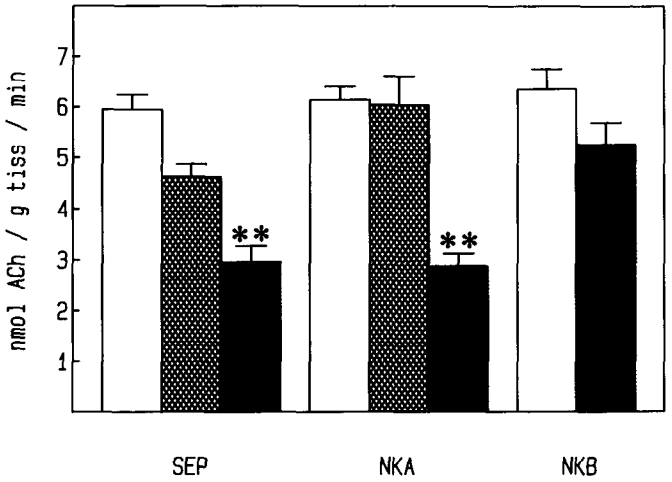

Figure 3. Effects of SPAF on endogenous ACh release evoked by TKs. No antagonist (*open bars*) or different concentrations of SPAF ([D-Arg^6, D-Trp^{7,9}, N-Methyl-Phe^8]-substance P fragment G11, 1 μM (*cross-hatched bars*) or 10 μM (*solid bars*), were injected into the detection medium containing the slice, 1 min before depolarization with 1 μM *SEP*, 0.1 μM *NKA*, or 0.01 μM *NKB*. Note that SPAF was more potent at antagonizing ACh release evoked by SEP than by NKA, and that it did not antagonize the effects of NKB. Each value corresponds to the mean ± SEM of four or more different experiments performed with duplicate or triplicate determinations. The statistical significance was evaluated by the Student's *t* test for nonpaired data with Bonferroni's correction for four comparisons. **, $P < 0.0025$ compared with the corresponding open bar.

to ACh release with the following rank order: $NK_3 > NK_2 > NK_1$.

TTX sensitivity of TK effects on cholinergic neurons

TTX has been often used to detect the participation of axonal transmission, which depends on fast Na^+ channels (Raitieri et al., 1984). However, in the case of neostriatal cholinergic interneurons, TTX cannot distinguish between activity mediated by polysynaptic paths and activity mediated by the dendrites and axons of the cholinergic neurons, because the entire cell is present in the preparation (Arenas et al., 1990a). In the present study, TTX blocked the excitatory effects of both SEP and NKA on endogenous ACh release from neostriatal slices, indicating that nerve conduction was involved in mediating the response. This is in agreement with the coexistence of NKA/SP and with the well-known SP input to the cell bodies and proximal dendrites of cholinergic interneurons (Bolam et al., 1986). Thus, the blockade of ACh release evoked by SEP and NKA is probably due to the TTX blockade of axonal transmission following somatodendritic activation of cholinergic neurons, as has been previously reported for ACh release evoked by glutamate (Scatton and Lehmann, 1982; Arenas et al., 1990a). Furthermore, lesion studies using kainate and 6-OHDA showed that SP binding sites in the neostriatum were mainly located on striatal cell bodies and dendrites rather than on afferent nerve terminals (Ritter et al., 1985; Mantyh and Hunt, 1986). These results have been confirmed by the TTX sensitivity of ^{3}H-dopamine release evoked by SP in striatal slices, which involves polysynaptic paths and suggests the absence of SP receptors on dopaminergic terminals (Petit and Glowinski, 1986).

The case of NKB is rather different, because TTX partially inhibited (50%) ACh release elicited by NKB. This observation suggests that NKB could partially act at both the somatodendritic field and nerve terminals of cholinergic neurons. It is very unlikely that the NKB effect at the somatodendritic field could be mediated by those NK receptors activated by SP or NKA, because SPAF antagonized the effects of SP and NKA but not those of NKB on NK_3 receptors. Alternatively, two populations of NKB-containing neurons acting on NK_3 receptors could be postulated. The first one, providing somatodendritic input to the cholinergic neurons, could be responsible for the TTX-sensitive effects on ACh release, and the second NKB population could provide input to the nerve terminals of cholinergic neurons, resulting in a TTX-resistant effect on ACh release.

Because SP terminals are in synaptic contact with the somatodendritic field of cholinergic neurons (Bolam et al., 1986) and SP evoked ACh release in a TTX-sensitive manner, it is very likely that the 59% of NKB mRNA–containing neurons that coexist with SP (Burgunder and Young, 1989) could correspond to the population of NKB neurons that provides TTX-sensitive input to the somatodendritic field of cholinergic neurons. In contrast, the 25% of NKB mRNA–containing neurons that coexist with enkephalin (Burgunder and Young, 1989) could correspond to the population of NKB neurons providing TTX-resistant input to the cholinergic terminals. This possibility is further supported by the TTX-resistant effects of δ-opioids on ACh release in the neostriatum (Arenas et al., 1990a) mediated by dopaminergic terminals (Arenas et al., 1991).

The dopaminergic modulation of the tachykininergic control of cholinergic neurons

Neurons containing SP have been reported to be the major target of nigrostriatal dopaminergic neurons in the head of the caudate nucleus (Beckstead, 1987). Furthermore, dopaminergic input to

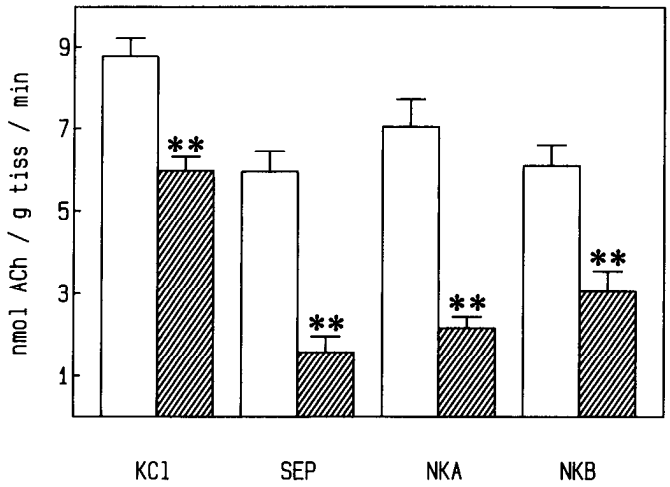

Figure 4. Effects of TTX on endogenous ACh release evoked by 50 mM *KCl*, 1 μM *SEP*, 0.1 μM *NKA*, or 0.01 μM *NKB* from rat neostriatal slices. *Open bars*, control ACh release; *hatched bars*, TTX (0.3 μM) diminished by 32% ACh release evoked by KCl, blocked by 70% ACh release evoked by SEP and NKA, and partially prevented (50%) ACh release evoked by NKB. Each value corresponds to the mean ± SEM of four different experiments performed with duplicate or triplicate determinations. The statistical significance was evaluated by the Student's *t* test for paired data. **, $P < 0.01$ compared with the corresponding open bar.

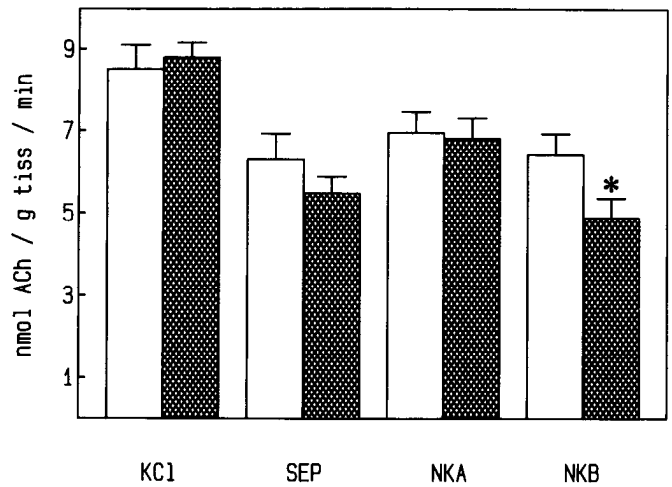

Figure 5. Effects of dopaminergic nigrostriatal deafferentation on endogenous ACh release evoked by 50 mM *KCl*, 1 μM *SEP*, 0.1 μM *NKA*, or 0.01 μM *NKB* from rat neostriatal slices. *Open bars*, ACh release evoked from the contralateral side to the 6-OHDA injection; *cross-hatched bars*, ACh release evoked from the ipsilateral side to the 6-OHDA injection, where only the effect of NKB was significantly reduced. Each value corresponds to the mean ± SEM of five different experiments performed with duplicate or triplicate determinations. The statistical significance was evaluated by the Student's *t* test for paired data. *, $P < 0.05$ compared with the corresponding open bar.

striatal neurons has been shown to modulate the biosynthesis and levels of many neurotransmitters or neuromodulators. Unilateral 6-OHDA lesions of the dopaminergic nigrostriatal pathway have shown two patterns of regulation. The first pattern is consistent with a downregulation, leading to a decrease in striatal SP levels (Voorn et al., 1987), SP mRNA (Young et al., 1986; Sivam et al., 1987), and dynorphin levels (Jiang et al., 1990). This downregulation was followed neither by the release of SP and NKA in the neostriatum (Lindefors et al., 1989) nor by altering the effects of SP and NKA on ACh release in the 6-OHDA–lesioned striatum (present results). Instead, the dopaminergic denervation decreased SP and NKA release in the substantia nigra (Lindefors et al., 1989). Thus, it is likely that the downregulation of SP levels (Voorn et al., 1987) and mRNA (Young et al., 1986; Sivam et al., 1987) in the neostriatum reflected the dopaminergic regulation of SP/NKA striatonigral neurons together with a masked or absent dopaminergic regulation of SP/NKA interneurons.

The second pattern of regulation of striatal neuronal activity is consistent with an upregulation of other neuronal systems, which leads to an increase in GABA levels (Lindefors et al., 1989), glutamic acid decarboxylase levels (Vernier et al., 1988), enkephalin mRNA (Young et al., 1986; Sivam et al., 1987; Vernier et al., 1988), enkephalin levels (Voorn et al., 1987; Jiang et al., 1990), and NKB mRNA (Burgunder and Young, 1989). These changes have also been reported to be followed by an increase in the release of some of these neurotransmitters such as GABA (Lindefors et al., 1989). Evidence in favor of the dopaminergic regulation of NKB release is still lacking; however, because both GABA and NKB exhibited an upregulation, it is reasonable to assume an increased release of NKB. Then, an increase in endogenous NKB release could lead to a desensitization of NK_3 receptors coupled to ACh release. This event is very likely responsible for the decreased response of cholinergic neurons to exogenously applied NKB after 6-OHDA lesions. Furthermore, desensitization of TK receptors by high concentrations of TK agonists has been reported (Laufer et al., 1988).

These findings suggest that the tonic dopaminergic influence on cholinergic neurons (Lehmann and Langer, 1983; Alberch et al., 1985) could be partially mediated by nerve terminals of NKB neurons acting on cholinergic terminals. This possibility is supported by the hyperactivity of NKB mRNA–containing neurons after the dopaminergic denervation (Burgunder and Young, 1989), by the dopaminergic regulation of NKB-evoked ACh release (present results), and by the partially TTX-resistant effect of NKB on ACh release (present results). Furthermore, the dopaminergic hyperactivity of the remaining terminals after the dopaminergic denervation (Zigmond et al., 1990) may be the result of the hyperactivity of NKB mRNA–containing neurons (Burgunder and Young, 1989).

Our results provide evidence favoring the idea that part of the dopaminergic regulation of cholinergic function can be mediated by NKB. This hypothesis suggests that the population of NKB neurons acting on cholinergic terminals could play an important role in the dopaminergic regulation of cholinergic neurons and in the regulation of neostriatal function. However, this hypothesis requires further testing, because NKB could also affect other neurotransmitter systems.

Implications for the basal ganglia disorders

Huntington's disease is characterized by the loss of small- and medium-sized spiny neurons containing GABA, enkephalin, and TKs (Kowal et al., 1987). In these patients, SP and NKA levels in caudate–putamen, globus pallidus, and substantia nigra are decreased, though NKB levels in the caudate–putamen and the external segment of the globus pallidus are unchanged (Arai et al., 1987). Interestingly, this spared pattern of NKB levels is coincident with the pattern described for striatal neurons expressing NKB mRNA (Burgunder and Young, 1989), which

possibly regulate neostriatal cholinergic neurons. These observations reinforce the idea of a differential regulation of SP/NKA- and NKB-containing neurons. Furthermore, these findings are in agreement with our hypothesis of a possible partial mediation by NKB terminals of the dopaminergic input to cholinergic neurons. The predominance of the dopaminergic neurotransmission in the neostriatum (Spokes, 1981) may lead to a tonic inhibition of NKB release from NKB-containing neurons and to a decrease in ACh release. If so, the enhancement of the NKB spared neurotransmission may help to restore the balance between ACh and dopamine.

In Parkinson's disease, there is a decrease in the striatal levels of dopamine (Hornykiewicz, 1982), an increase in the striatal levels of GABA (Perry et al., 1983), and a decrease in the contents of SP in the substantia nigra and external globus pallidus, but not in the caudate–putamen or the internal globus pallidus (Agid and Javoy-Agid, 1985). Unfortunately, no data are available concerning modifications of NKB levels in Parkinson's disease; however, two opposite patterns of dopaminergic regulation of striatal neurons can be observed as described for 6-OHDA lesions. Therefore, in agreement with the mediation of the dopaminergic input to cholinergic neurons by NKB, a possible increase in the release of NKB in Parkinson's disease and 6-OHDA lesions may be responsible for the predominance of the cholinergic neurotransmission in the neostriatum. If this is true, NKB antagonists or NKB-depleting therapy, acting on a spared population of neurons and at the next step of the dopaminergic input, may have a role in the control of cholinergic function and in the recovery of motor symptoms in Parkinson's disease.

Further work and highly selective NKB antagonists are required to determine fully the role of the NKB system in the dopaminergic input to cholinergic neurons and in basal ganglia disorders.

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