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Short communication

Local application of SCH 39166 reversibly and dose-dependently decreases acetylcholine release in the rat striatum

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Abstract

The effect of local application by reverse dialysis of the dopamine D_1 receptor antagonist (−)-*trans*-6,7,7*a*,8,9,13*b*-exahydro-3-chloro-2-hydroxy-*N*-methyl-5*H*-benzo-[*d*]-naphto-[2,1*b*]-azepine hydrochloride (SCH 39166) on acetylcholine release was studied in awake, freely moving rats implanted with concentric microdialysis probes in the dorsal striatum. In these experiments, the reversible acetylcholine esterase inhibitor, neostigmine, was added to the perfusion solution at two different concentrations, 0.01 and 0.1 μM. SCH 39166 (1, 5 and 10 μM), in the presence of 0.01 μM neostigmine, reversibly decreased striatal acetylcholine release (1 μM SCH 39166 by 8 ± 4%; 5 μM SCH 39166 by 24 ± 5%; 10 μM SCH 39166 by 27 ± 7%, from basal). Similarly, SCH 39166, applied in the presence of a higher neostigmine concentration (0.1 μM), decreased striatal acetylcholine release by 14 ± 4% at 1 μM, by 28 ± 8% at 5 μM and by 30 ± 5% at 10 μM, in a dose-dependent and time-dependent manner. These results are consistent with the existence of a facilitatory tone of dopamine on striatal acetylcholine transmission mediated by dopamine D_1 receptors located on striatal cholinergic interneurons.

Keywords: Acetylcholine; SCH 39166; Microdialysis; Striatum; Dopamine D_1 receptor

1. Introduction

Striatal cholinergic neurons synapse with medium-size spiny neurons over large territories of the striatum and receive a dopaminergic input from the substantia nigra–ventral tegmental area (Lehmann and Langer, 1983; Kawaguchi, 1992; Di Chiara et al., 1994). Dopamine is currently thought to control in a reciprocal fashion acetylcholine neurotransmission in the striatum via D_1-like and D_2-like receptors, stimulating acetylcholine release by dopamine D_1 receptors and inhibiting it by dopamine D_2 receptors (Consolo et al., 1987, 1992, 1996; Bertorelli and Consolo, 1990; Damsma et al., 1990a,b, 1991; Abercrombie and DeBoer, 1997; Acquas et al., 1997).

Still, an issue that awaits clarification is that of the anatomical location of dopamine D_1 receptors controlling acetylcholine release in the striatum. Thus, although changes have been reported by different groups after local infusion of dopamine D_1 agonists and antagonists in the striatum (Anderson et al., 1994; Consolo et al., 1992, 1996), these observations have not been confirmed by others (Damsma et al., 1991; DeBoer et al., 1992; Acquas et al., 1997).

These discrepancies have been addressed by Consolo et al. (1996) who showed that the inhibitory effects of the dopamine D_1 receptor antagonist SCH 23390, locally applied by reverse dialysis, on striatal acetylcholine release are dependent on rat strain and on the anaesthetic used during surgery for probe implant. The aim of the present study was to further clarify this issue by studying the effect of the selective dopamine D_1 antagonist, (−)-*trans*-6,7,7*a*,8,9,13*b*-exahydro-3-chloro -2- hydroxy-*N*-methyl-5*H*-benzo-[*d*]-naphto-[2,1*b*]-azepine hydrochloride (SCH 39166) (Chipkin et al., 1988), that in contrast to SCH 23390 has a low affinity for $5HT_2$ receptors, on striatal acetylcholine release after local application by reverse dialysis. In addition, given the suggestion that the effects of dopamine agonists and antagonists on acetylcholine

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release are related to the concentration of the acetylcholine esterase inhibitor (neostigmine) in the Ringer (DeBoer and Abercrombie, 1996), we also studied the effect of SCH 39166 at two different concentrations (0.01 and 0.1 μM) of neostigmine in the perfusion Ringer.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (275–300 g) were housed in groups of two to three per cage for at least 3 days before use and were maintained on a 12/12 h light/dark cycle (lights on at 0730 h) with food and water available ad libitum. One day after the surgical procedure for the implant of the microdialysis probe, rats were individually housed in hemispherical bowls, which also served as the experimental environment. Experiments were carried out between 0900 and 1600 h at least 24–30 h after the surgical implant.

2.2. Surgery and microdialysis

Rats were anaesthetized with ketamine HCl (Ketalar, Parke Davis, Italy) (100 mg/kg i.p.) and stereotaxically implanted with a concentric microdialysis probe in the dorsal striatum. The coordinates, measured from bregma, were AP = +0.7 mm, DV = −6.5 mm, ML = −3.0, according to the atlas of Paxinos and Watson (1986).

The membrane used was a polyacrylonitrile/sodium methallyl sulphonate copolymer (AN 69, Hospal, France). The membrane was covered with epoxy glue along its whole length except for 3 mm corresponding to the area of dialysis. The day of the experiment rats were connected to a microperfusion pump by polyethylene tubing connected to a 2.5-ml glass syringe containing the perfusion solution. The perfusion flow was set at 1 μl/min. Samples were collected every 10 min into a 20 μl sample loop and subsequently injected in a HPLC injector valve. The perfusion solution contained 147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$, in twice distilled water. To achieve consistently detectable amounts of acetylcholine in the dialysate, the reversible acetylcholine esterase inhibitor, neostigmine bromide (0.01 and 0.1 μM) (Sigma, MO, USA), was added to the perfusion solution. Acetylcholine was assayed by high-pressure liquid chromatography coupled with electrochemical detection (HPLC–ECD) in conjunction with an enzyme reactor (Damsma et al., 1987). Acetylcholine was separated on a reverse phase Chromspher C_{18} 5 μm (Merck, FRG) column (75 × 2.1 mm). The mobile phase passed directly through the enzyme reactor containing acetylcholine esterase (ED 3.1.1.7; type VI-S, Sigma) and choline oxidase (EC 1.1.3.17; Sigma) covalently bound to glutaraldehyde-activated Lichrosorb 10-NH_2; acetylcholine was quantitatively converted into hydrogen peroxide which was detected electrochemically at a platinum working electrode set at 500 mV vs. an Ag/AgCl reference electrode (LC-4B, BAS, IN, USA). The mobile phase was an aqueous potassium phosphate buffer (1.9 mM K_2HPO_4, 0.2 mM tetramethyl ammonium hydroxide, pH = 8) delivered at a constant flow of 0.4 ml/min by an HPLC pump. The detection limit of the assay was about 50 fmol/sample. Injections of an acetylcholine standard (20 μl, 0.1 μM) were made every 60 to 90 min in order to monitor changes in electrode sensitivity and sample concentrations were corrected accordingly.

2.3. Drugs

SCH 39166 (Schering-Plough, Milan, Italy) was dissolved in Ringer containing neostigmine 0.01 or 0.1 μM.

2.4. Statistics

Values are expressed as percent changes with respect to baseline (100%). Baseline was set as the average of the last six pretreatment samples, not differing more than 15%. One-way, two-way and three-way analysis of variance (ANOVA), with time as the repeated measure, were used to analyze the treatment effects. Tukey's post hoc analyses were applied for multiple comparisons, with the statistical significance set at $P < 0.05$.

3. Results

3.1. Basal striatal acetylcholine output

Basal acetylcholine (fmol/min), was calculated and defined as the average ± S.E.M. of the six pretreatment samples for each experimental group. The overall mean ± S.E.M. baseline of acetylcholine was 38 ± 3 fmol/min ($n = 27$) in the presence of neostigmine 0.01 μM and 97 ± 4 fmol/min ($n = 31$) in the presence of neostigmine 0.1 μM in the perfusion Ringer.

3.2. Effect of local perfusion with SCH 39166 on striatal acetylcholine release

Fig. 1 shows the effect of local administration of the dopamine D_1 receptor antagonist SCH 39166 on striatal acetylcholine release in presence of neostigmine 0.01 μM (upper panel) and 0.1 μM (lower panel) in the perfusion Ringer. As shown in Fig. 1, upper panel, at neostigmine 0.01 μM, SCH 39166 1 μM failed to affect acetylcholine release ($F(12,72) = 0.9$, NS), while at 5 and 10 μM, SCH 39166 reversibly reduced acetylcholine output in the striatum to 76 ± 5% and 73 ± 7% of baseline, respectively. One-way ANOVA with the dose of SCH 39166 as the independent variable showed that SCH 39166 significantly affected acetylcholine output with respect to baseline,

$F(12,60) = 2.31$, $P < 0.016$ and $F(12,96) = 4.76$, $P < 0.0001$, respectively, for SCH 39166 5 and 10 μM. Two-way ANOVA with time as the within subjects factor and the concentrations of SCH 39166 as the between-subjects factor yielded a significant effect of concentration ($F(3,24) = 7.54$, $P < 0.001$); Tukey's post hoc analysis demonstrated a significant difference between SCH 39166 5 and 10 μM with respect to SCH 39166-free Ringer and to SCH 39166 1 μM.

Fig. 1, lower panel, shows the effect of the application of SCH 39166 (0, 0.5, 1, 5, and 10 μM) on striatal acetylcholine output in presence of neostigmine 0.1 μM in the Ringer. SCH 39166 dose-dependently and reversibly decreased striatal acetylcholine release; thus, while perfusion with SCH 39166-free Ringer and SCH 39166 0.5 μM failed to affect this measure ($F(6,36) = 1.1$, NS and $F(12,72) = 0.5$, NS), concentrations of SCH 39166 of 1, 5, and 10 μM decreased acetylcholine output from baseline maximally to $86 \pm 4\%$, $72 \pm 8\%$ and $70 \pm 5\%$, respectively. One-way ANOVA with time as the within subjects factor provided significant F ratios, $F(12,60) = 4.49$, $P < 0.0001$, $F(12,60) = 4.7$, $P < 0.0001$ and $F(12,60) = 8.3$, $P < 0.0001$ with respect to baseline for SCH 39166 1, 5 and 10 μM, respectively. Two-way ANOVA with the concentration of SCH 39166 as the independent variable showed a significant effect of dose ($F(4,26) = 7.29$, $P < 0.0004$), time ($F(11,286) = 6.85$, $P < 0.00001$) and time × dose interaction ($F(44,286) = 1.99$, $P < 0.0004$). Tukey's post hoc analysis showed a significant difference between SCH 39166 5 μM and 10 μM as compared to SCH 39166-free Ringer and SCH 39166 0.5 μM.

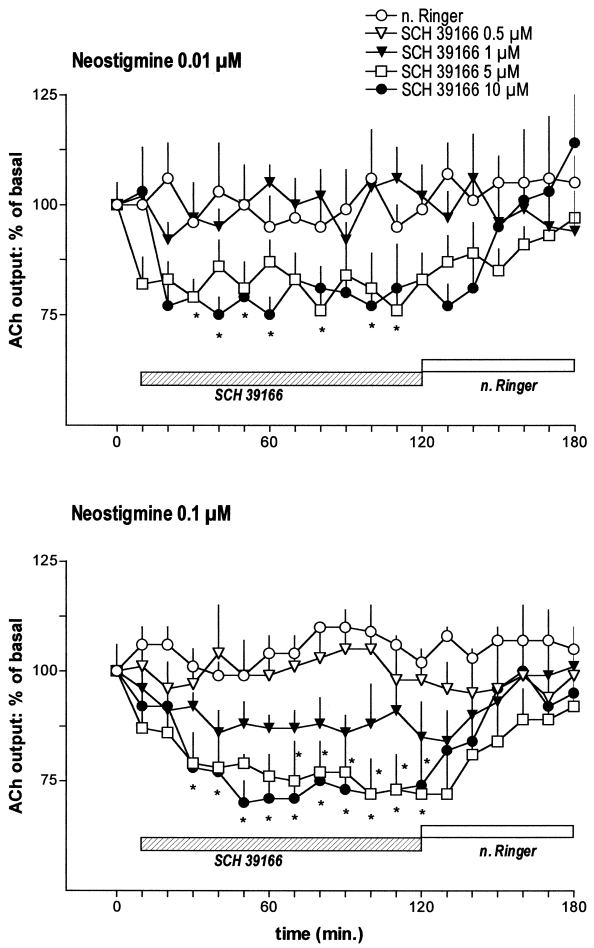

Fig. 1. (Upper panel) Effects of SCH 39166 (0, 1, 5, and 10 μM) ($n = 5$, 7, 6, and 9, respectively), on striatal acetylcholine release in presence of neostigmine 0.01 μM in the perfusion Ringer. Horizontal bars indicate the application of SCH 39166 at the different concentrations and of SCH 39166-free Ringer. Values are expressed as percentage baseline. Vertical bars represent S.E.M. $^*P < 0.05$ with respect to the corresponding time point of SCH 39166-free Ringer group.
(Lower panel) Effects of SCH 39166 (0, 0.5, 1, 5, and 10 μM) ($n = 7$, 7, 5, 6, and 6, respectively), on striatal acetylcholine release in presence of neostigmine 0.1 μM in the perfusion Ringer. Horizontal bars indicate the application of SCH 39166 at the different concentrations and of the SCH 39166-free Ringer. Values are expressed as percentage baseline. Vertical bars represent S.E.M. $^*P < 0.05$ with respect to the corresponding time point of SCH 39166-free Ringer group.

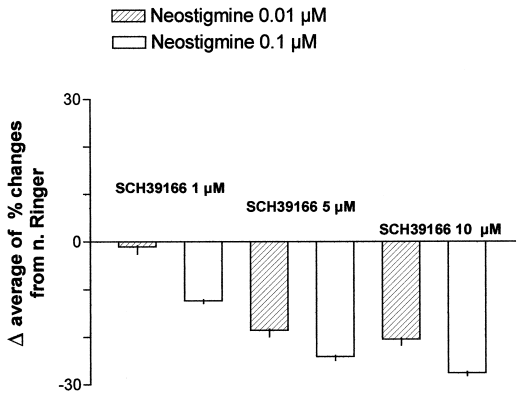

Fig. 2. Difference between the effects of SCH 39166 (1, 5, and 10 μM) in presence of 0.01 and 0.1 μM neostigmine and the correspondent time points during perfusion with SCH 39166-free Ringer, normalized to 100%. Data are the average of the last 80 min during SCH 39166 perfusion.

Fig. 2 shows the changes in striatal acetylcholine release at steady-state, i.e., during the last 80 min of SCH 39166 perfusion at 1, 5, and 10 μM in the presence of neostigmine 0.01 and 0.1 μM, expressed as Δ score % with respect to the values of SCH 39166-free Ringer. Three-way ANOVA, with treatment (SCH 39166 0, 1, 5, and 10 μM) and acetylcholine esterase inhibitor concentration (neostigmine 0.01 and 0.1 μM) as the between-subjects factor and time (the last height samples of SCH 39166 perfusion) as the within subjects factor did not show a significant interaction ($F(21,301) = 0.75$, NS).

4. Discussion

The present study shows that dialysis perfusion of the dorsal striatum with SCH 39166 (1–10 μM) reduces

acetylcholine release in a concentration-dependent and time-dependent fashion. These effects were quantitatively similar at two neostigmine concentrations, 0.01 and 0.1 μM. These observations therefore confirm, by the use of a close structural analog, SCH 39166, the observations of Consolo et al. (1992, 1996) obtained with SCH 23390. Furthermore, the results of the present study extend those of Consolo et al. (1992, 1996) on two aspects: that of the specificity, being SCH 39166, in contrast to SCH 23390, devoid of affinity for $5HT_2$ receptors (Bishoff et al., 1986; Chipkin et al., 1988; Bijak and Smialowski, 1989) and that of the reversibility, as acetylcholine release recovered to basal levels when the Ringer containing SCH 39166 was replaced with SCH 39166-free Ringer.

The reduction of acetylcholine release after intrastriatal application of SCH 39166 by the dialysis probe contrasts with the negative results of Acquas et al. (1997) obtained at concentrations of SCH 39166 higher (50 μM) than those used in the present study (1–10 μM). This discrepancy, in light of the observations of Consolo et al. (1996), might be related to differences in rat strain (Sprague–Dawley vs. Wistar) and general anaesthetic (ketamine vs. pentobarbital). Therefore, the present study is consistent with the conclusions of Consolo et al. (1996) on the critical importance of specific experimental conditions for obtaining a decrease of acetylcholine release after local application of a dopamine D_1 receptor antagonist in the rat striatum.

On the basis of differences in the response to amphetamine and to the dopamine D_1 receptor agonist SKF 38393 in relation to the concentration of neostigmine in the Ringer, DeBoer and Abercrombie (1996) have suggested that the stimulatory influence of dopamine D_1 receptors on striatal acetylcholine release is a function of acetylcholine esterase inhibition in the area around the dialysis probe. This conclusion, however, has been questioned by Di Chiara et al. (1996). These authors note that the changes in the amount of D_1-stimulated release at different neostigmine concentrations in the Ringer observed by DeBoer and Abercrombie (1996) were associated to changes in the basal release of acetylcholine. Thus, recalculation of the data of DeBoer and Abercrombie (1996) as percent of basal release, shows that the increase of acetylcholine release by D_1 receptor stimulation is independent from neostigmine concentrations in the Ringer (38%, 35% and 35% at 0, 0.01 and 0.1 μM neostigmine, respectively). This conclusion is consistent with that of Acquas and Fibiger (1998) who studied the effects of neostigmine concentrations on A-77636-induced increases of acetylcholine release and failed to obtain differences when results are expressed as percent of basal acetylcholine release (Acquas and Fibiger, 1998). The present study shows that also the effects of a dopamine D_1 receptor antagonist on acetylcholine release are independent from neostigmine concentrations. Results similar to the present ones have been obtained by us after systemic administration of SCH 39166 (Acquas and Di Chiara, 1999).

In conclusion, the results of the present study corroborate the hypothesis of the existence of a tonic stimulatory influence of dopamine on striatal acetylcholine release mediated by striatal dopamine D_1 receptors. This hypothesis has been recently strengthened by the finding that most if not all striatal acetylcholine interneurons express messenger RNA for dopamine D_1-like receptors (Bergson et al., 1995; Jongen-Rêlo et al., 1995; Yan et al., 1997) and by electrophysiological evidence that dopamine D_1 receptor stimulation is excitatory on striatal cholinergic neurons (Aosaki et al., 1998).

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References

Abercrombie, E.D., DeBoer, P., 1997. Substantia nigra D_1 receptors and stimulation of striatal cholinergic interneurons by dopamine: a proposed circuit mechanism. J. Neurosci. 17 (21), 8498–8505.

Acquas, E., Di Chiara, G., 1999. D_1 receptor mediated control of striatal acetylcholine release by endogenous dopamine. Eur. J. Pharmacol. 382, 121–127.

Acquas, E., Fibiger, H.C., 1998. Dopaminergic regulation of striatal acetylcholine release: the critical role of acetylcholinesterase inhibition. J. Neurochem. 70 (3), 1088–1093.

Acquas, E., Wilson, C., Fibiger, H.C., 1997. Nonstriatal dopamine D1 receptors regulate striatal acetylcholine release in vivo. J. Pharmacol. Exp. Ther. 281 (1), 360–368.

Anderson, J.J., Kuo, S., Chase, T.N., Engberg, T.M., 1994. Dopamine D_1 receptor-stimulated release of acetylcholine in rat striatum is mediated indirectly by activation of striatal neurokinin-1 receptors. J. Pharmacol. Exp. Ther. 269, 1144–1151.

Aosaki, T., Kiuchi, K., Kawaguchi, Y., 1998. Dopamine D_1-like receptor activation excites rat striatal large aspiny neurons in vitro. J. Neurosci. 18 (14), 5180–5190.

Bergson, C., Mrzljak, L., Smiley, J.F., Pappy, M., Levenson, R., Goldman-Rakic, P.S., 1995. Regional, cellular, and subcellular variations in the distribution of D_1 and D_5 dopamine receptor subtypes in primate brain. J. Neurosci. 15 (12), 7821–7836.

Bertorelli, R., Consolo, S., 1990. D_1 and D_2 dopaminergic regulation of acetylcholine release from striata of freely moving rats. J. Neurochem. 54, 2145–2148.

Bijak, M., Smialowski, A., 1989. Serotonin receptor blocking effect of SCH 23390. Pharmacol. Biochem. Behav. 32, 585–587.

Bishoff, S., Heinrich, M., Sonntag, J.M., Krauss, J., 1986. The D1 dopamine receptor antagonist SCH 23390 also interacts with brain serotonin ($5\text{-}HT_2$) receptors. Eur. J. Pharmacol. 129, 367–370.

Chipkin, R.E., Iorio, L.C., Coffin, V.L., McQuade, R.D., Berger, J.G., Barnett, A., 1988. Pharmacological profile of SCH 39166: a dopamine D_1 selective benzonaphtazepine with potential antipsychotic activity. J. Pharmacol. Exp. Ther. 247 (3), 1093–1102.

Consolo, S., Wu, C.F., Fusi, R., 1987. D-1 receptor-linked mechanism modulates cholinergic neurotransmission in rat striatum. J. Pharmacol. Exp. Ther. 242 (1), 300–305.

Consolo, S., Girotti, P., Russi, G., Di Chiara, G., 1992. Endogenous dopamine facilitates striatal in vivo acetylcholine release by acting on D1 receptors localized in the striatum. J. Neurochem. 59, 1555–1557.

Consolo, S., Colli, E., Caltavuturo, C., Di Chiara, G., 1996. Surgical anaesthesia with pentobarbital prevents the effect of local SCH 23390 on rat striatal acetylcholine release in a strain-dependent manner. Behav. Pharmacol. 7, 663–668.

Damsma, G., Westerink, B.H.C., De Vries, J.B., Van den Berg, C.J., Horn, A.S., 1987. Measurement of acetylcholine release in freely moving rats by means of automated intracerebral microdialysis. J. Neurochem. 48, 1523–1528.

Damsma, G., DeBoer, P., Westerink, B.H.C., Fibiger, H.C., 1990a. Dopaminergic regulation of striatal cholinergic interneurons: an in vivo microdialysis study. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 523–527.

Damsma, G., Tham, C.-S., Robertson, G.S., Fibiger, H.C., 1990b. Dopamine D_1 receptor stimulation increases striatal acetylcholine release in the rat. Eur. J. Pharmacol. 186, 335–338.

Damsma, G., Robertson, G.S., Tham, C.-S., Fibiger, H.C., 1991. Dopaminergic regulation of striatal acetylcholine release: importance of D1 and *N*-Methyl-D-Aspartate receptors. J. Pharmacol. Exp. Ther. 259 (3), 1064–1072.

DeBoer, P., Abercrombie, E.D., 1996. Physiological release of striatal acetycholine In Vivo: modulation by D1 and D2 dopamine receptor subtypes. J. Pharmacol. Exp. Ther. 277 (2), 775–783.

DeBoer, P., Damsma, G., Schram, Q., Stoof, J.C., Zaagsma, J., Westerink, B.H.C., 1992. The effect of intrastriatal application of directly and indirectly acting dopamine agonists and antagonists on the in vivo release of acetylcholine measured by brain microdialysis. Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 144–152.

Di Chiara, G., Morelli, M., Consolo, S., 1994. Modulatory functions of neurotransmitters in the striatum: ACh/Dopamine/NMDA interactions. TINS 17, 228–233.

Di Chiara, G., Tanda, G., Carboni, E., 1996. Estimation of in-vivo neurotransmitter release by brain microdialysis: the issue of validity. Behav. Pharmacol. 7, 640–657.

Jongen-Rêlo, A., Docter, G.J., Jonker, A.J., Voorn, P., 1995. Differential localization of mRNAs encoding dopamine D_1 or D_2 receptors in cholinergic neurons in the core and shell of the rat nucleus accumbens. Mol. Brain Res. 28, 169–174.

Kawaguchi, Y., 1992. Large aspiny cells in the matrix of the rat neostriatum in vitro: physiological identification, relation to the compartments and excitatory post-synaptic currents. J. Neurophysiol. 67, 1669–1682.

Lehmann, J., Langer, S.Z., 1983. The striatal cholinergic interneuron: synaptic target of dopaminergic terminals? Neuroscience 10 (4), 1105–1120.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. 2nd edn. Academic Press, Australia.

Yan, Z., Song, Y.Z., Surmeier, J., 1997. D2 dopamine receptors reduce N-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. J. Neurophysiol. 77 (2), 1003–1015.