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Sodium nitroprusside stimulates L-DOPA release from striatal tissue through nitric oxide and cGMP

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Abstract

The effects of the nitric oxide (NO) donor, sodium nitroprusside, on L-DOPA and dopamine release from striatal tissue were evaluated using a static incubation system in which the striatal tissue released between three and six times more L-DOPA than DA, although the DA content was four times higher than that of L-DOPA. Sodium nitroprusside stimulated L-DOPA release in a time- and concentration-dependent (25, 50 and 100 μM) manner. This effect was not due to an increase in L-DOPA synthesis because sodium nitroprusside did not modify the tyrosine hydroxylase activity of striatal tissue. DA release was also stimulated by sodium nitroprusside but it required a higher concentration (500 μM) and longer incubation (60 min). Neither basal nor sodium nitroprusside-stimulated L-DOPA release was influenced by Ca^{2+} deprivation (EGTA 5 mM) and/or the presence of nitrendipine (1 μM), a blocker Ca^{2+} channel, in the incubation medium. However, cGMP (1 mM) increased L-DOPA release, and the soluble guanylate cyclase inhibitor, 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) (5 μM), partially blunted the stimulatory effect of sodium nitroprusside 100 μM. In addition, the presence of certain scavengers of free radicals, such as uric acid (300 μM) or melatonin (300 μM) but not of superoxide dismutase (1000 UI/ml) or salicylic acid (300 μM), completely blocked sodium nitroprusside (100 μM)-induced L-DOPA release. These results show that NO stimulates L-DOPA release from striatal tissue by an apparently Ca^{2+}-independent mechanism, mediated by cGMP but also by peroxynitrite.

Keywords: c-GMP; Dopamine; L-DOPA (L-3,4-dihydroxyphenylalanine); Nitric oxide (NO); Peroxynitrite; Striatum

1. Introduction

L-3,4-Dihydroxyphenylalanine is proposed as a neurotransmitter and/or neuromodulator in the central nervous system (CNS), based on the finding that, under in vitro and in vivo experimental conditions, it is released in a transmitter-like manner, and it produces various presynaptic and postsynaptic actions (Misu et al., 1996). Additionally, neurons containing L-DOPA as an end-product have been detected in various parts of the CNS. In vivo and in vitro studies of the striatum, an important target region for L-DOPA, have reported Ca^{2+}-dependent and tetrodotoxin-sensitive release of L-DOPA (Goshima et al., 1988; Nakamura et al., 1992). This suggests that this catecholamine is released via a depolarization-dependent process, although from some compartment other than dopamine-containing vesicles (Misu et al., 1996).

Nitric oxide (NO) is involved in a wide range of physiological roles in the CNS including the modulation of neurotransmitter release (Szabó, 1996). NO primarily operates via activation of a soluble guanylate cyclase and cGMP-dependent modulation of several intracellular processes (Garthwaite and Boulton, 1995). Moreover, under physiological conditions, NO can react with superoxide to form peroxynitrite, which decomposes spontaneously to generate a hydroxyl radical (Huie and Padjama, 1993; Darley-Usmar and Hallibel, 1996). Thus, it is possible that some of the actions of NO may also be mediated by peroxynitrite and/or other free radicals.

In the striatum, NO is produced from a small population of neurons that contain both somatostatin and neuropeptide Y (Vincent and Johannson, 1983) and is involved in the release of several neurotransmitters including dopamine, 5-hydroxytryptamine (5-HT), acetylcholine and γ-aminobutyric acid (GABA) (Zhu and Luo, 1992; Guevara-Guzman et

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al., 1994; Pogun et al., 1994; Serra et al., 2000; Trabace and Kendrick, 2000). However, its possible role in L-DOPA release has not been widely studied. The aim of the present study is to examine the possible involvement of NO in L-DOPA release from striatal fragments incubated in vitro, its possible mediators and mechanisms of action.

2. Materials and methods

Sodium nitroprusside, superoxide dismutase, uric acid, melatonin, salicylic acid, and 8-Br-cGMP were obtained from Sigma, Madrid. 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) was supplied by Tocris Cookson.

The experiments were carried out according to institutional and international policies in compliance with the European Communities Council Directive (86/609/EEC). Adult male rats of the Sprague–Dawley strain weighing 260–280 g were kept under controlled conditions of temperature (22 ± 1 °C) and a 12:12-h light–dark cycle (lights on at 08:00 h), with free access to rat chow and water. On the day of the experiments, the animals were killed by decapitation, their brains were quickly removed, lightly frozen and the corpus striata were dissected and cut into small pieces of about 2 mg. Each piece of striatal tissue was re-suspended in 500 μl of Dulbecco's modified Eagle's medium pre-warmed to 37 °C and equilibrated in an atmosphere of 5% CO_2 and 95% air. After pre-incubation for 60 min, media were replaced and tissues were incubated for 30 or 60 min under the same conditions, with medium alone or containing the substances to be tested. At the end of the incubation, media were collected and frozen for the measurement of dopamine and L-DOPA concentrations by high performance liquid chromatography (HPLC) according to previously reported techniques (González et al., 1998) and [NO_2^-] by a fluorimetric assay (Misko et al., 1993). Under these experimental conditions, striatal tissue, which contained four times more DA than L-DOPA, nevertheless released three to six times more L-DOPA than DA (data not shown).

Preliminary experiments were performed to evaluate the time course of nitrite release into the medium (as an index of NO production) from striatal tissue incubated in the presence and absence of sodium nitroprusside, and incubation periods of 30 and 60 min were used. Nitrites were routinely measured, as a control, in all experiments.

Tissue viability was assessed by measuring lactate released into the medium from striatal tissue incubated in the absence or presence of 100 and 500 μM of sodium nitroprusside for 30 and 60 min. Lactate was measured in duplicate 100-μl aliquots of media using a standard colorimetric assay provided by Sigma (Sigma Kit 735). Lactate release per minute was not significantly modified either by the presence of sodium nitroprusside or by a longer incubation time with respect to control incubations (60 min), indicating that the effects of sodium nitroprusside, at doses used in this study, were be not due to non-specific cell damage. Sodium nitroprusside was dissolved immediately before use and protected from light. Scavengers were added to the incubation buffers immediately before sodium nitroprusside, and ODQ was present in pre-incubation and incubation media.

Results are expressed as means ± S.E.M. with significant differences determined by one-way analysis of variance (ANOVA) using the Tukey post hoc test.

3. Results

The NO generator sodium nitroprusside stimulated in a concentration-dependent manner, in the range of 25 to 100 μM, L-DOPA release from striatal fragments incubated for 30 (ANOVA, $P<0.01$) and 60 min (ANOVA, $P<0.05$), as is

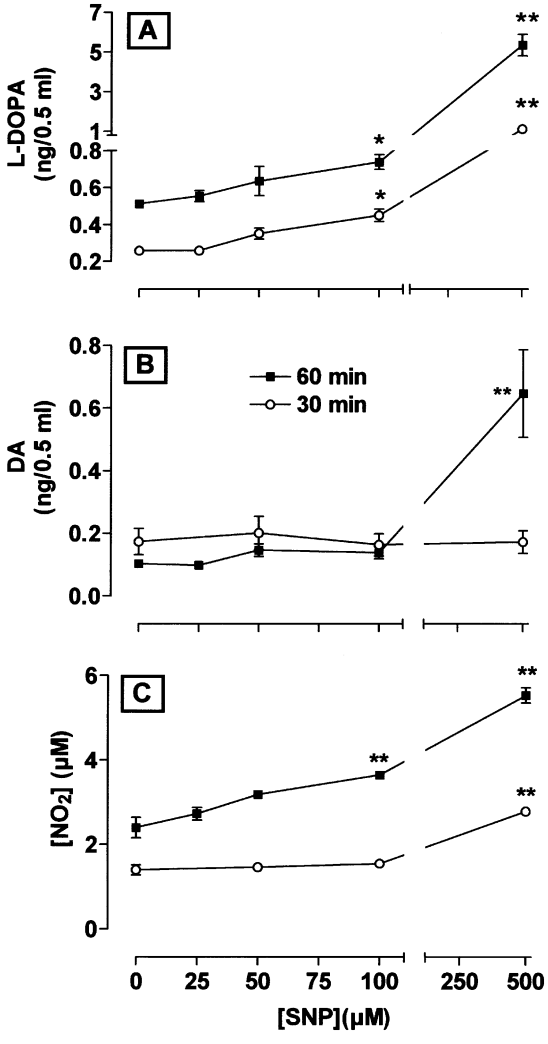

Fig. 1. Effects of increasing concentrations of the NO donor sodium nitroprusside (SNP) on L-DOPA (A), dopamine (B) and [NO_2^-] (C) released from striatal tissue fragments incubated in vitro. Data represent means ± S.E.M. * $P<0.05$, ** $P<0.001$.

shown in Fig. 1A. The highest concentration of sodium nitroprusside (500 μM) also significantly increased L-DOPA release from striatal tissue (Fig. 1A). Sodium nitroprusside in the range of 25 to 100 μM had no effect on DA release after either 30 min or 60 min of incubation; however, it significantly stimulated DA release at a concentration of 500 μM when the incubation time was prolonged to 60 min (Fig. 1B). This effect of sodium nitroprusside on L-DOPA release was not due to an increase in its synthesis because the NO donor did not modify the tyrosine hydroxylase activity of striatal slices incubated for 1 h (results not shown). Fig. 1C shows the $[NO_2^-]$ released from striatal slices incubated for 30 and 60 min with sodium nitroprusside.

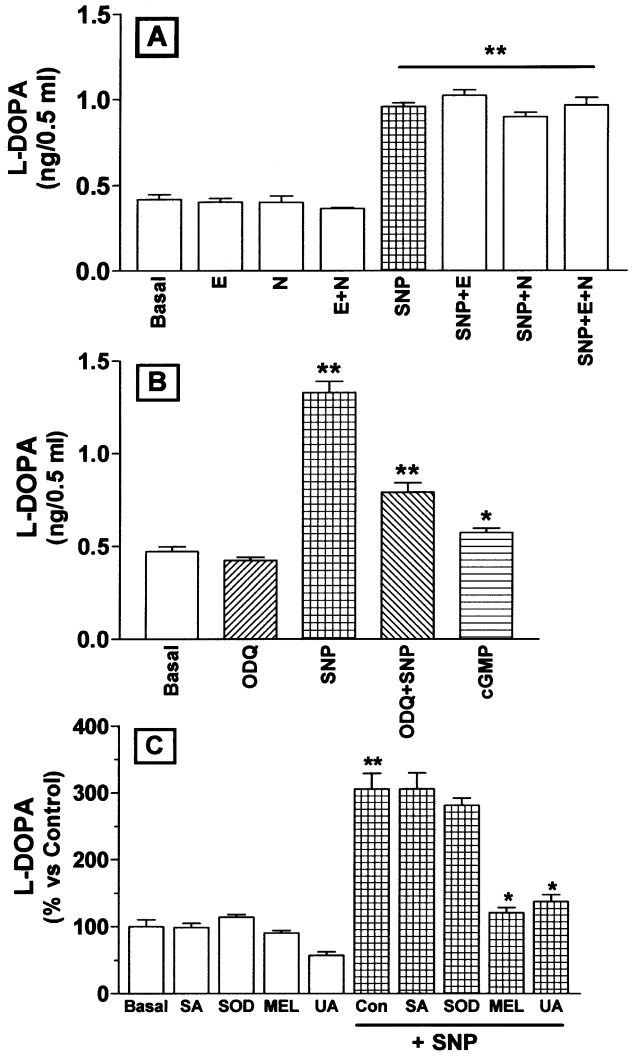

Fig. 2. (A) Effect of the presence of EGTA (E) 5 mM and/or nitrendipine (N) 1 μM on basal and SNP 100 μM-stimulated L-DOPA release. (B) Effect of cGMP (1 mM) and the guanylate cyclase inhibitor ODQ (5 μM) on basal and SNP (100 μM)-stimulated L-DOPA release from striatal tissue fragments incubated for 1 h. Data represent means ± S.E.M. (C) Effect of the presence in the incubation medium of different free radical scavengers (SA 300 μM, SOD 1000 U/ml, MEL 300 μM, UA 300 μM) on basal and SNP-induced L-DOPA release. Data are presented as a percentage of control of each experiment and represent means ± S.E.M. * $P<0.05$, ** $P<0.001$.

The releasing effect of sodium nitroprusside on striatal L-DOPA was not influenced by addition of EGTA 5 mM and/or nitrendipine 1 μM, an antagonist of L-type Ca^{2+} channel, to the incubation buffer, as is shown in Fig. 2A. However, the effect of sodium nitroprusside seemed to be dependent on the cGMP/GC system, at least in part: the addition of 8-Br-cGMP (1 mM) to the incubation medium slightly increased ($P<0.05$) basal L-DOPA release, and the presence of 5 μM of 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ), a guanylyl cyclase inhibitor, partially blunted the L-DOPA release stimulated by sodium nitroprusside 100 μM without affecting its basal release ($P<0.001$)(Fig. 2B).

In order to evaluate the possible involvement of other free radicals in the L-DOPA-releasing effect of sodium nitroprusside, the action of sodium nitroprusside 100 μM was assessed in the presence of several scavengers including superoxide dismutase, a superoxide anion scavenger; uric acid, a peroxynitrite scavenger; melatonin, which has been reported to detoxify hydroxyl radical, peroxynitrite anion, singlet oxygen and nitric oxide; and salicylic acid, a hydroxyl radical scavenger. Both uric acid (300 μM) and melatonin (300 μM) completely blunted sodium nitroprusside-induced L-DOPA release without having any effect on its basal release, as is shown in Fig. 2C. However, the addition of superoxide dismutase (1000 U/ml) or salicylic acid (300 μM) to the incubation medium did not modify either basal or sodium nitroprusside-stimulated L-DOPA release (Fig. 2C). The presence of scavengers in the incubation buffer did not significantly modify $[NO_2^-]$ release, except in the case of melatonin, which reduced sodium nitroprusside $[NO_2^-]$ production.

4. Discussion

The major findings of the present study are as follows. (a) The NO generator sodium nitroprusside increased in a time- and concentration-dependent way L-DOPA release from striatal fragments incubated in vitro. This effect was not due to an increase in L-DOPA synthesis because sodium nitroprusside did not modify the tyrosine hydroxylase activity of striatal tissue. DA release was also stimulated by sodium nitroprusside but it required higher concentrations. (b) L-DOPA release from striatal incubations was not modified by EGTA and/or nitrendipine; however, cGMP increased it, and the presence of the soluble guanylate cyclase inhibitor ODQ partially blunted the stimulatory effect of sodium nitroprusside. (c) Scavengers of free radicals, such as uric acid or melatonin but not superoxide dismutase or salicylic acid, completely blocked sodium nitroprusside induced L-DOPA release.

NO has been implicated in the modulation of the release of several neurotransmitters from different brain areas (Szabó, 1996). In the striatum, it has been reported that in

vivo microdialysis of NO donors, *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) or (*Z*)-1-[2-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (NOC-18), increases the release of acetylcholine, 5-hydroxytryptamine, glutamate, GABA and taurine (Guevara-Guzman et al., 1994; Trabace and Kendrick, 2000). Concerning DA release, in vivo and in vitro studies have reported that NO generators either increase (Zhu and Luo, 1992; West and Galloway, 1997; Iravani et al., 1998; Serra et al., 2000) or decrease it (Guevara-Guzman et al., 1994; Trabace and Kendrick, 2000). However, the possible involvement of NO in L-DOPA release has been poorly studied. To our knowledge, only one report (Serra et al., 2000) has examined the effects of the NO donors, sodium nitroprusside and 3-morpholino sydnonimine (SIN-1), on DA and L-DOPA release from striata of freely moving rats using brain microdialysis. These authors found that intrastriatal infusion of sodium nitroprusside greatly increased the dialysate DA concentration and decreased the L-DOPA concentration; however, SIN-1 increased DA release while L-DOPA release was unaffected. The present results show that in vitro sodium nitroprusside stimulates both L-DOPA and DA release although with different potency. L-DOPA release seems to be 10 times more sensitive to NO than DA release.

Neither the basal nor sodium nitroprusside-induced release of L-DOPA from striatal tissue was affected by Ca^{2+} deprivation and/or the Ca^{2+} channel blocker nitrendipine, suggesting that L-DOPA is released from a non-vesicular compartment presumably cytosolic, by an apparently Ca^{2+}-independent mechanism. Goshima et al. (1988) suggested that L-DOPA release from striatal slices could be due to activation of a carrier-mediated efflux of amino acids which is unaffected or potentiated by Ca^{2+} omission. Instances of Ca^{2+}-independent DA release have also been reported. Amphetamine induces Ca^{2+}-independent DA release from striatal slices, presumably from a extravesicular or cytoplasmic DA pool (Ofori et al., 1986), and L-DOPA induces DA release by a process that does not involve either Ca^{2+}-dependent exocytosis or reverse transport. (Snyder and Zigmond, 1990).

The L-DOPA-releasing action of sodium nitroprusside seems to be mediated by the cGC/cGMP signaling system, but also by peroxynitrite: cGMP stimulated L-DOPA release from striatal tissue, and the presence of the sGC inhibitor ODQ in the incubation medium partially blunted sodium nitroprusside-induced L-DOPA release. The addition of uric acid, a peroxynitrite scavenger, or melatonin, which has been reported to detoxify hydroxyl radical, peroxynitrite anion, singlet oxygen and nitric oxide (Reiter et al., 1999), but not salicylic acid, a hydroxyl radical scavenger, or superoxide dismutase, an anion superoxide-scavenging enzyme, also completely blocked NO donor-stimulated L-DOPA release. The lack of effect of superoxide dismutase to block the action of sodium nitroprusside could be related to its inability to cross cell membranes to avoid peroxynitrite synthesis. The major intracellular target of NO in the brain is soluble guanylate cyclase (Ignarro, 1991), which when activated produces an increase in cGMP concentration in target cells and modulation of the activity of cGMP-dependent enzymes, ion channels, etc, but it has also been demonstrated that under physiological conditions NO can react with superoxide to generate peroxynitrite (Huie and Padjama, 1993), and that some of the actions of NO may actually be mediated by this powerful oxidant. It has been reported that peroxynitrite mediates the acetylcholine release evoked by sodium nitroprusside and SNAP from primary cultured cerebral cortex neurons (Ohkuma et al., 1995) and the aspartate release from rat cerebellar granule cells. Also, the inhibition of DOPA synthesis caused by SIN-1 in PC12 cells (Ischiropoulos et al., 1995) and the inhibition of tyrosine hydroxylase activity in rat median eminence by sodium nitroprusside (Abreu et al., 2000) seem to be mediated by peroxynitrite. The release of acetylcholine, 5-HT, GLU and GABA induced by SNAP and NOC-18 from the striatum has been reported to be mediated through cGMP-dependent mechanisms; however, it has been reported that NO can inhibit DA release through peroxynitrite formation and increase it through sGC/cGMP (Trabace and Kendrick, 2000). The present results indicate that both mechanisms also modulate L-DOPA release from the striatum, but in this case, they both move in the same direction, stimulating L-DOPA release.

In summary, our results show that NO is an important modulator of L-DOPA release from the striatum. It stimulates L-DOPA release in a time- and concentration-dependent way by a Ca^{2+}-independent mechanism. The action of NO on L-DOPA release is mediated not only by cGMP but also by peroxynitrite formation.

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