Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 395 (2006) 138–142
Neuroscience Letters
www.elsevier.com/locate/neulet

α_2-Adrenoceptor-mediated modulation of the release of GABA and noradrenaline in the rat substantia nigra pars reticulata

Amal Alachkar [a], Jonathan Brotchie [b], Owen T. Jones [a,*]

[a] *Faculty of Life Sciences, University of Manchester, 1.124 Stopford Building, Oxford Road, Manchester M13 9PT, UK*
[b] *Division of Neuroscience, Toronto Western Hospital Research Institute, Toronto, Canada M5T 2S8*

Received 11 August 2005; received in revised form 21 October 2005; accepted 26 October 2005

Abstract

The control of movement by the basal ganglia is influenced by inputs from diverse brain structures. Unfortunately, the mechanisms of modulation are poorly defined. Based on neuroanatomical evidence for α_{2A} and α_{2C} subtypes of α_2 adrenergic receptors within this region, we hypothesize that noradrenergic α_2-receptors can influence transmitter release in the SNr. To test this hypothesis we examined the effect of the alpha 2 adrenergic agonist, clonidine, and antagonist, rauwolscine, on the efflux of [^{3}H]-GABA and [^{3}H]-noradrenaline from brain slices of the rat substantia nigra pars reticulata. At low concentrations (10 nM), rauwolscine caused an $84.2 \pm 18.51\%$ ($p < 0.01$) increase in KCl-evoked GABA release. At higher concentrations, rauwolscine caused a dose-dependent return to basal levels. Rauwolscine also enhanced basal GABA efflux after KCl washout with a similar biphasic concentration-dependence. Surprisingly, clonidine also enhanced [^{3}H]-GABA release but had no effect on KCl-evoked [^{3}H]-GABA release at concentrations which inhibited [^{3}H]-NA efflux. These effects were potentiated by the GABA re-uptake inhibitor nipecotic acid. Together, our data indicate an important role for noradrenergic modulation in the SNr. The enhancing effect of both the α_2 adrenoceptor agonist and antagonist on GABA release, while appearing paradoxical, can be rationalised by actions at distinct subsets of α_2 adrenoceptors, using a simple model where α_{2A} adrenoceptors are localized on the terminals of noradrenergic afferents impinging upon α_{2C} adrenoceptor-containing GABAergic striato-nigral neurones.

Keywords: GABA; Noradrenaline; Release; Alpha 2 adrenergic receptor; Substantia nigra; Rat brain

There is now compelling evidence that multiple neurotransmitters are involved in the control of movement. This is especially true in the substantia nigra pars reticulata (SNr), which, together with the internal segment of the globus pallidus, represents the output nuclei of the basal ganglia. While the primary transmitter in neurons projecting from the SNr (to the thalamus) is GABA, the SNr itself receives GABAergic inputs from the striatum [11]. Such inputs can be modulated by diverse neurotransmitters including dopamine [18], glutamate [21], and serotonin [22], released directly from afferent projections to the SNr or indirectly, from within the striatum.

Over the last two decades, converging lines of evidence have suggested that noradrenaline plays an important modulatory role in the substantia nigra [1]. First, autoradiographic studies have revealed significant binding of adrenergic ligands in the SNr [2,25]. Second, immunocytochemical studies have demonstrated the presence of α_2 adrenoceptors in the SNr [19]. Third, noradrenaline uptake sites have been identified in the SNr [5]. Fourth, there is anatomical evidence for an interaction between noradrenergic neurons in the locus coeruleus and the substantia nigra [15].

Whether, and by what mechanism, noradrenaline could modulate GABA release in the SNr is uncertain. While α_1 but not β-adrenergic receptors enhance excitability in the SNr [1], a role for α_2 adrenergic receptors in modulating the release of other neurotransmitters in other areas of the brain is well established. Thus, several studies have shown that activation of α_2 adrenoceptors by noradrenaline inhibits glutamate [17], acetylcholine [23], dopamine [6], and serotonin [12], release in various regions of the brain. Conversely, in the rat hippocampus, cerebral cortex and striatum noradrenaline has a facilitatory effect on the release of GABA from the nerve terminals [16,27].

Unfortunately, in spite of its potential modulatory actions, the actual role of noradrenaline in the basal ganglia remains poorly

* Corresponding author. Tel.: +44 161 275 5604; fax: +44 161 275 5363.
E-mail address: owen.t.jones@manchester.ac.uk (O.T. Jones).

0304-3940/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2005.10.069

defined. Nevertheless, we hypothesize that noradrenergic input, acting via α_2 adrenergic receptors, is a key modulator of GABA release within the SNr. To test this hypothesis we have examined the effect of an α_2 agonist, clonidine, and an α_2 antagonist, rauwolscine, on basal and KCl-evoked release of GABA in brain slices of the SNr.

Our data indicate that increasing concentrations of rauwolscine act in a biphasic fashion such that low concentrations (<10 nM) caused a significant increase whereas high concentrations caused a return to basal levels of KCl-evoked GABA release from the SNr. While clonidine had no effect on KCl-evoked GABA release from the SNr, it had a small significant effect on basal release that again showed a biphasic dependence on concentration. These effects were potentiated by the GABA re-uptake inhibitor nipecotic acid. Together, our data indicate an important role for noradrenergic modulation in the SNr.

Rats were purchased from Manchester University, BSU. Clonidine, rauwolscine, desipramine, pargyline and nipecotic acid were purchased from Sigma, UK. [^{3}H]-GABA and [^{3}H]-noradrenaline (NA) were from NEN, UK. Filter disks were from Whatman International Ltd.

All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Male Sprague–Dawley rats (250–350 g) were killed by inhalation of an overdose of halothane. The brains were rapidly transferred to 4 °C artificial cerebrospinal fluid (aCSF: 118 mM NaCl, 4.8 lM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM $KHPO_4$, 11 mM glucose, 0.6 mM ascorbic acid) and coronal (400 μm) tissue slices taken. The SNr was dissected from surrounding tissue using a stainless steel tube (1.5 mm diameter) sharpened at one end.

Slices from the SNr were incubated at 37 °C for 30 min in 2 ml of aerated aCSF (95% O_2/5% CO_2) pH 7.4, containing either 100 nM [^{3}H]-GABA (NEN, specific activity 81 Ci/mmol) or 400 nM [^{3}H]-NA (NEN, specific activity 13.7 Ci/mmol). The slices were then placed in individual chambers of a Brandel SF-12 superfusion System (Brandel Instrument, Gaithersburg, USA) sandwiched between filter discs and perfused at 37 °C with oxygenated aCSF at a flow rate of 0.5 ml/min. For assays of [^{3}H]-NA release, the monoamine oxidase inhibitor pargyline, 10 μM and the NA uptake inhibitor desipramine (10 μM), were added throughout the experiment. Slices were perfused for 30 min to allow [^{3}H]-GABA or [^{3}H]-NA efflux to stabilize and the perfusate was then collected in 5-min time bins. At the end of the experiment, tassue slices were re-suspended in 0.5 ml aCSF, disrupted by probe sonication (Brandelin Sonoplus HD70, Brandelin Electronic, Berlin, Germany). The radioactivity in the perfusate or sonicated tissue was then determined by adding 0.5 ml aliquots to 4.5 ml of scintillation fluid and liquid scintillation counting (Packard Instrument Co. Illinois, USA). Isotope release was expressed as the fractional rate of release (FRR), which was equal to the radioactivity released during a given 5 min period divided by the total radioactivity present in the slice at the beginning of that same 5 min period.

KCl-evoked release of [^{3}H]-GABA and [^{3}H]-NA was assessed by applying a 5 min pulse of aCSF containing 40 mM KCl and was calculated by subtracting the treatment and vehicle FRR profiles for the duration of the KCl pulse. Since [^{3}H]-GABA efflux in the post-KCl pulse period cannot be quantified through subtraction of treatment and vehicle peak profiles, it was estimated from the FRR at a fixed time point $FRR_{t=x\ \mathrm{min}}$.

To examine the effect of an α_2 adrenoceptor agonist, antagonist, and GABA uptake inhibitor, tissues were perfused throughout the experiment with aCSF containing clonidine, rauwolscine, or nipecotic acid, respectively.

One brain slice was taken from each coronal section and six from each animal. In all experiments between five and seven animals were used. Data is shown as mean ± standard error of the mean (S.E.M.) for (n) independent experiments. Since uncertainty in the underlying mechanism precluded curve-fitting to specific models, dose–response profiles were examined using one-way analysis of variance (ANOVA) with Dunnett correction for comparisons made against vehicle and Tukey's test for multiple treatment comparisons. Time-courses for basal and evoked release were analysed by two-way ANOVA with Bonferroni correction.

Perfusion of SNr slices with the antagonist rauwolscine, at 10 nM, induced a significant increase (84.2 ± 18.51%; $p < 0.01$) in KCl-evoked [^{3}H]-GABA efflux when compared to vehicle (Fig. 1A). At this concentration, rauwolscine also caused a gradual and increasingly significant rise in the efflux of [^{3}H]-GABA in the period following the KCl pulse (Fig. 1A).

Analysis of the effect of rauwolscine on the KCl-evoked efflux of [^{3}H]-GABA revealed a biphasic dependence on concentration (Fig. 1B) with significant elevations at 10 nM (84.2 ± 18.51%; $p < 0.01$; above) and 100 nM (51.6 ± 16.5%; $p < 0.05$) when compared to vehicle. Lower or higher concentrations of rauwolscine had no statistically significant effect on KCl-evoked [^{3}H]-GABA efflux when analysed, similarly. The efflux of [^{3}H]-GABA in the post-KCl period (calculated as $FRR_{t=70}$; the FRR at 70 min (35 min post-KCl pulse), showed an identical biphasic-dependence on rauwolscine concentration as KCl-evoked [^{3}H]-GABA efflux did. Thus, the post-KCl basal efflux was elevated significantly at 10 nM ($FRR_{t=70}$ 16.7 ± 1.48%; $p < 0.01$) and 100 nM ($FRR_{t=70}$ 17.1 ± 1.8%; $p < 0.01$) but not higher or lower concentrations ($p > 0.05$; data not shown) when compared to vehicle controls ($FRR_{t=70}$ 9.4 ± 0.95%).

The agonist, clonidine, at 10 μM, like rauwolscine, caused a significant increase in [^{3}H]-GABA efflux when compared to vehicle controls during the experimental time-course (Fig. 1C). However, unlike rauwolscine, clonidine had no significant effect on KCl-evoked efflux of [^{3}H]-GABA, specifically, when compared to vehicle controls ($p > 0.05$; Fig. 1D). The efflux of [^{3}H]-GABA in the post-KCl period (calculated as $FRR_{t=65}$ above) showed a significant elevation at 10 μM ($FRR_{t=65}$ 9.9 ± 0.5%; $p < 0.01$) but not at lower (1 μM) or higher (100 μM) concentrations ($p > 0.05$; data not shown) when compared to vehicle controls ($FRR_{t=65}$ 8 ± 0.3%).

Nipecotic acid is reported to block the uptake of released GABA [9] and has a more defined role than other presynaptic GABA-ergic modulators such as GABA-B receptor agonists in the SnR [26]. We, therefore, tested whether nipecotic acid might unmask, or potentiate, any effects of rauwolscine