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Tyramine excites rat subthalamic neurons in vitro by a dopamine-dependent mechanism

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Abstract

Tyramine, an endogenous ligand for mammalian trace amine-associated receptors, may act as a neuromodulator that regulates neuronal activity in basal ganglia. Using whole-cell patch recordings of subthalamic nucleus (STN) neurons in rat brain slices, we found that bath application of tyramine evoked an inward current in voltage-clamp in over 60% of all STN neurons. The inward current induced by tyramine was mimicked by the D_2-like dopamine receptor agonist quinpirole, but was only partially blocked by the D_2-like receptor antagonist sulpiride. In contrast, the D_1-like receptor agonist SKF38393 evoked no current in STN neurons. Inward current evoked by tyramine was significantly reduced by the catecholamine uptake inhibitor nomifensine, and by exhausting catecholamines in the brain via pretreatment with reserpine. Tyramine also reduced the amplitude of $GABA_A$ receptor-mediated IPSCs that were evoked by focal electrical stimulation of the slice. Inhibition of IPSCs by tyramine was mimicked by quinpirole and was blocked by sulpiride but not by SCH23390, a D_1 receptor antagonist. Moreover, tyramine-induced inhibition of IPSCs was reduced in slices pretreated with reserpine, and this inhibition could be restored by briefly superfusing the slice with dopamine. These results suggest that tyramine acts as an indirect dopamine agonist in the STN. Although inhibition of IPSCs is mediated by D_2-like receptors, the dopamine-dependent inward currents evoked by tyramine do not fit a typical dopamine receptor pharmacological profile.

Keywords: Tyramine; Dopamine; Subthalamic neurons; Inward current; Synaptic current; Subthalamic nucleus; Brain slices; Trace amine; Electrophysiology

1. Introduction

Trace amines are a family of compounds that are chemically related to classical biogenic monoamines. In general, trace amines are produced by decarboxylation of those amino acids that serve as precursors for the classical monoamines dopamine, noradrenaline, and serotonin (Berry, 2004). Consequently, it is not surprising that trace amines and their binding sites are more abundant in monoamine-containing cells such as locus ceruleus and substantia nigra, as well as in areas of the brain that receive monoaminergic innervation, such as the basal ganglia (Durden and Davis, 1993; Vaccari, 1986; Boulton et al., 1977). Although trace amines normally exist in brain at relatively low concentration, levels of trace amines are increased in a variety of human conditions. For example, trace amine levels are greatly increased by levodopa that is used in the treatment of Parkinson's disease (Edwards et al., 1981). Also, antidepressant agents that inhibit monoamine oxidase slow the metabolic degradation of trace amines and thereby increase levels of trace amines in brain (Branchek and Blackburn, 2003). So even though trace amines are not likely to be stored and released as classical neurotransmitters, there is growing appreciation that these substances may play important roles in human disease (Premont et al., 2001).

Tyramine is a trace amine that is structurally similar to dopamine. This structural similarity makes tyramine a good substrate for both the cytoplasmic dopamine transporter (Sitte et al., 1998;

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Parker and Cubeddu, 1988) and the vesicular monoamine transporter-2 (Partilla et al., 2006). Disruption of these transport mechanisms by tyramine causes release of dopamine into the extracellular space (Vaccari et al., 1991). Interest in tyramine has grown in recent years since it was suggested that tyramine might be an endogenous ligand for a newly cloned family of trace amine receptors (TARs) (Borowsky et al., 2001; Bunzow et al., 2001). These receptors are G protein-coupled, and their activation is positively coupled to adenylyl cyclase. Trace amines tyramine and β-phenylethylamine bind with nanomolar affinity to the TAR_1 subtype, and its mRNA is widely expressed at low levels throughout the brain (Borowsky et al., 2001). Although the physiological consequence of TAR activation is not known, there is some evidence that TAR activation can alter G protein-dependent second messenger systems (Federici et al., 2005). However, pharmacological characterization of TARs has been hampered by the lack of selective antagonists.

Our laboratory has a long-standing interest in the role of dopamine in regulating neuronal activity in the subthalamic nucleus (STN). The STN is composed of glutamate-containing neurons that exert a strong excitatory influence on the major output nuclei of the basal ganglia (Parent and Hazrati, 1995). As a result, the STN is a key basal ganglia structure for the control of normal and abnormal movement. In Parkinson's disease, the loss of dopamine causes STN neurons to exhibit excessive burst firing of action potentials, which is thought to contribute to symptoms of this disease (Bergman et al., 1998; Guridi and Obeso, 1998). Studies by our lab and elsewhere have shown that dopamine excites STN neurons by evoking an inward current that is mediated by a reduction in a resting K^+ conductance (Mintz et al., 1986; Zhu et al., 2002a,b). However, it is thought that this excitatory effect of dopamine might benefit parkinsonism because membrane depolarization can convert the firing of action potentials from a bursting pattern to that of a single-spike firing pattern (Beurrier et al., 1999). In addition, dopamine has been shown to act presynaptically to inhibit GABA-mediated synaptic transmission in the STN (Shen and Johnson, 2000; Cragg et al., 2004). Based upon our previous experience with dopamine in the STN, we were interested in extending our studies to characterize possible dopamine-like actions of a trace amine such as tyramine.

Therefore, we undertook the present study to characterize possible dopaminergic actions of tyramine on membrane properties and synaptic currents in STN neurons. A secondary goal was to identify possible non-dopaminergic actions of tyramine that might be mediated by the newly described TAR. Although our results largely support the conclusion that tyramine is an indirect dopamine agonist, our data also suggest that some actions of tyramine may follow an unconventional pharmacological profile.

2. Materials and methods

2.1. Tissue preparation

Midbrain slices were prepared from male Sprague–Dawley rats (140–250 g; Simonsen, Gilroy, CA), as described previously (Shen and Johnson, 2000). Animals used in this study were treated in accordance with Institutional Guidelines and the National Institutes of Health (USA) regarding the care and use of animals for experimental procedures. Briefly, rats were anesthetized with isoflurane and sacrificed by severing major thoracic vessels. The brain was rapidly removed, and horizontal slices (300 μm thick) containing caudal diencephalon and rostral mesencephalon were cut in cold physiological saline with a vibratome (Series 1000, Technical Products International Inc., St. Louis, MO). A slice containing the STN was then transferred to a recording chamber (volume 500 μl) and immobilized with an electron microscopy grid. The slice was submerged and superfused (1.5–2 ml/min) with a standard extracellular solution (aCSF) containing (in mM): NaCl, 126; KCl, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 2.4; glucose, 11; $NaHCO_3$, 21. This solution was saturated with 95% O_2 and 5% CO_2, and had a pH 7.35 at 35–36 °C. Using a dissection microscope for visual guidance, the STN was located as grey matter about 2.7 mm lateral to the midline and 2.0 mm rostral from the center of the substantia nigra pars reticulata (Paxinos and Watson, 1986).

2.2. Dopamine depletion

In some experiments, brain slices were superfused with the tyrosine hydroxylase inhibitor α-methyl-DL-*p*-tyrosine (AMPT, 30 μM) for at least 3 h before recording in order to deplete dopamine from the cytosolic pool. This treatment interferes with effects of dopamine releasing agents such as amphetamine, as has been shown previously by Mercuri et al. (1989). In other experiments, we also used reserpine in order to interfere with vesicular storage of dopamine (Carlsson, 1975). Rats were given an injection of reserpine (5 mg/kg i.p.) 24 h prior to the preparation of brain slices. Thereafter, slices were superfused with 10 μM reserpine and 30 μM AMPT.

2.3. Electrophysiological recordings

Slices were allowed to recover for 1–2 h before starting recordings. "Blind" patch-clamp recordings in whole-cell configuration were conducted in STN neurons with pipettes that had a resistance of 6–8 MΩ when filled with (in mM): K-gluconate, 135; NaCl, 10; $CaCl_2$, 1; EGTA, 10; HEPES, 10; MgATP, 2; Na_3GTP, 0.5 (pH 7.3, 290–300 mOsmol/l). Recordings were made in voltage- or current-clamp mode with an Axopatch-1D amplifier and Digidata 1200 digitizer controlled with pClamp 9 software (Molecular Devices, Sunnyvale, CA, USA). Holding currents were measured at −70 mV recorded continuously using a MacLab digitizer controlled with Chart software (AD Instruments, Colorado Springs, CO, USA). Series resistance was monitored intermittently and data were discarded if values changed by more than 20%. Membrane potentials were corrected for the liquid junction potential (−10 mV).

2.4. Synaptic currents

Bipolar stimulation electrodes (tip separation 300–500 μm) were placed in the slice immediately rostral (100–200 μm) to the STN to evoke synaptic responses. A single rectangular pulse (0.1 ms duration) of constant current (0.1–1 mA) was delivered every 10 s. The amplitude of evoked synaptic currents was measured from the average of three responses. An IPSC mediated by $GABA_A$ receptors was isolated pharmacologically by recording in the presence of (±)-2-amino-5-phosphonopentanoic acid (AP5, 25 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) in order to block ionotropic glutamate receptors. The IPSC was confirmed as being mediated by $GABA_A$ receptors by blocking the response with the antagonist bicuculline methiodide (BMI, 30 μM).

2.5. Drug application

All drugs added to the superfusate were first dissolved as aqueous stock solutions with the exception of CNQX, sulpiride and nomifensine, which were dissolved in dimethyl sulfoxide (DMSO). Each stock solution was diluted at least 1:1000 to the desired concentration in superfusate immediately prior to its use. DMSO, diluted 1:1000 in superfusate, had no effect on either