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Trace Amines Depress $GABA_B$ Response in Dopaminergic Neurons by Inhibiting G-$\beta\gamma$-Gated Inwardly Rectifying Potassium Channels

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ABSTRACT

Trace amines (TAs) are present in the central nervous system in which they up-regulate catecholamine release and are implicated in the pathogenesis of addiction, attention-deficit/hyperactivity disorder, Parkinson's disease, and schizophrenia. By using intracellular and patch-clamp recordings from dopaminergic cells in the rat midbrain slices, we report a depressant postsynaptic action of two TAs, β-phenylethylamine (β-PEA) and tyramine (TYR) on the $GABA_B$-mediated slow inhibitory postsynaptic potential and baclofen-activated outward currents. β-PEA and TYR activated G-proteins, interfering with the coupling between $GABA_B$ receptors and G-$\beta\gamma$-gated inwardly rectifying potassium channels. This is the first demonstration that β-PEA and TYR depress inhibitory synaptic potentials in neurons of the central nervous system, supporting their emerging role as neuromodulators.

Trace amines are biological compounds previously believed to have a marginal role in regulating neuronal functions. They are present at low levels in mammalian neuronal tissue and are packaged and released along with traditional amines (Boulton, 1976, 1982; Durden and Philips, 1980; Parker and Cubeddu, 1988). They have been considered to be "false transmitters", which displace active biogenic amines from their stores and act on catecholamine transporters in an amphetamine-like manner (Janssen et al., 1999; Mundorf et al., 1999). A specific role for TAs was suggested by the discovery of two subtypes of G-protein–coupled TA receptors, TA_1 and TA_2; both functionally coupled to increases in cAMP formation (Borowsky et al., 2001; Bunzow et al., 2001), presumably via the $G\alpha_s$ class of G-proteins. However, the physiological actions of these receptors are poorly understood, and research efforts so far have been confined to reconstituted in vitro expression systems. Considering that mRNA for the TA_1 and TA_2 receptors is present in the substantia nigra/ventral tegmental area (VTA) (Borowsky et al., 2001) and cyclic AMP-dependent protein kinase A (PKA) facilitates $GABA_B$ currents (Cameron and Williams, 1993; Bonci and Williams, 1996; Couve et al., 2002), we postulated that the biogenic amines β-PEA and TYR could increase the evoked $GABA_B$ slow inhibitory postsynaptic potential (IPSP) in midbrain dopaminergic neurons. Our electrophysiological experiments unexpectedly demonstrated that β-PEA and TYR reduce the $GABA_B$ IPSP by a G-protein–coupled postsynaptic mechanism. This depressant effect was mediated by neither PKA nor protein kinase C (PKC)-dependent phosphorylation nor by phospholipase C (PLC) activation, but it results mainly from TA-activated G-proteins interfering with the opening of $GABA_B$-gated GIRK channels. Together, our re-

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ABBREVIATIONS: TA, trace amine; β-PEA, β-phenylethylamine; TYR, tyramine; GIRK, G-$\beta\gamma$-gated inwardly rectifying potassium; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; DA, dopamine; VTA, ventral tegmental area; SNc, substantia nigra pars compacta; ACSF, artificial cerebrospinal fluid; IPSP, inhibitory postsynaptic potential; EPSP, excitatory postsynaptic potential; I-V, current-voltage relationship; IBac, baclofen-activated outward current; $PtIns(4,5)P_2$, phosphatidylinositol-3,4-biphosphate; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; U73122, 1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1*H*-pyrrole-2,5-dione; DHPG, (*S*)-3,5- dihydroxyphenylglycine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; SCH23390, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; AP-5, 2-amino-5-phosphonopentanoic acid; CGP55845, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl]phenylmethyl phosphinic acid; SQ22,536, 9-(tetrahydro-2′-furyl)adenine; Rp-cAMPS, 3′,5′-cyclic monophosphorothioate, *Rp*-isomer.

sults reveal a new role for β-PEA and TYR in depressing $GABA_B$ synaptic transmission. Given that this phenomenon occurs in dopaminergic cells, it is relevant to the control of brain operations linked to movement, cognition, and motivation.

Materials and Methods

Slice Preparation. Adult male Wistar rats (150–250 g) were used for all experiments. Standard procedures were used to obtain slices of the ventral midbrain for electrophysiological recordings (Mercuri et al., 1995). All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Briefly the brain was dissected and mounted on an agar block, and horizontal slices (250 μm) were cut by a vibratome at 8 to 10°C. Slices were maintained in artificial cerebrospinal fluid (ACSF) for 45 min at 33°C in an interface chamber before being transferred to a superfusing recording chamber. The ACSF contained 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 10 mM glucose, and 19 mM $NaHCO_3$, pH 7.4, equilibrated with a mixture of 95% O_2/5% CO_2.

Intracellular Recordings. Intracellular recordings from midbrain substantia nigra pars compacta (SNc) and VTA dopaminergic neurons were performed at 35°C in a recording chamber in which the slice was immobilized with two titanium grids and perfused at a rate of 2.5 to 3 ml/min with ACSF solution. Neurons were identified as dopaminergic by their electrical properties that included the presence of a regular spontaneous firing activity, a relaxation of the hyperpolarizing electrotonic potentials mediated by the activation of I_h, and the $GABA_B$ IPSP (Grace and Onn, 1989; Johnson and North, 1992; Mercuri et al., 1995). Because no differences were observed between cells of the VTA and SNc, the data were pooled. To prevent spontaneous spikes, the membrane potential was adjusted between −65 and −70 mV by hyperpolarizing current injection. The recording electrodes were filled with 2 M KCl and had a tip resistance of 30 to 80 MΩ. Synaptic events were evoked with bipolar tungsten-stimulating electrodes located within the slice and having a tip separation of 300 to 600 μm. The $GABA_B$ IPSP was evoked by a train of four to eight stimuli of 70 μs at 8 to 20 V delivered at 70 Hz every 30 s (Johnson et al., 1992). The amplitude of synaptic potentials was measured from traces representing the average of four responses. All $GABA_B$ synaptic potentials were recorded in the presence of a pharmacological cocktail containing bicuculline methiodide (50 μM), strychnine (1 μM), prazosin (300 nM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), 2-amino-5-phosphonopentanoic acid (AP-5; 50 μM), SCH23390 (3–10 μM), and sulpiride (1–3 μM) to block $GABA_A$, glycine, α_1-adrenergic, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, *N*-methyl-D-aspartate, and D_1- and D_2-like receptors, respectively. In some experiments, the $GABA_B$ receptor antagonist CGP55845 (300 nM) was perfused to block the $GABA_B$ IPSP. The $GABA_A$ IPSP was evoked by a single electrical stimulus with the same cocktail of drugs used to evoke the $GABA_B$ potential but omitting bicuculline. The fast excitatory postsynaptic potential (EPSP) was obtained by a single stimulus with the same cocktail of drugs used to evoke the $GABA_B$ potential but omitting CNQX and AP-5.

Patch-Clamp Recordings. Whole-cell patch-clamp recordings were obtained with an amplifier (Axopatch 200B; Axon Instruments Inc., Union City, CA) from visually and electrophysiologically identified dopamine (DA) neurons in the SNc and VTA using 1.5-mm patch pipettes (3–4 MΩ) made from borosilicate glass (WPI, Sarasota, FL) and pulled with a PP83 puller (Narishige, Tokyo, Japan). The electrophysiological characteristics were published previously (Liss et al., 1999; Tozzi et al., 2003). Membrane currents were digitized at 5 kHz through a Digidata 1200B A/D converter, acquired, and analyzed using pClamp software (Axon Instruments). Pipettes were filled with a standard internal solution containing 145 mM K-gluconate, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 0.75 mM EGTA, 2 mM Mg_2-ATP, 0.3 mM Na_3-GTP, or 145 mM potassium methylsulfate, 8 mM KCl, 10 mM HEPES, 2 mM Mg-ATP, and 0.3 mM Na_3GTP, pH 7.35 with KOH. The extracellular perfusate consisted of ACSF plus tetrodotoxin (1 μM), sulpiride (1–3 μM), and SCH23390 (3–10 μM). Access-resistance was monitored at regular intervals. A 10-mV hyperpolarizing step (250 ms) was applied to measure membrane resistance. The current-voltage relationship was measured with a step protocol (1 s per sweep, four sweeps, −60 to −120 mV) executed after the GIRK conductance was completely activated (GTPγS + baclofen) and in the presence of the trace amine at steady-state. All membrane potentials were corrected for the calculated liquid junction potential (13–15 mV).

Ca^{2+} Imaging. The fluorescent ion indicator Fura-2 was loaded into the cell via the patch pipette, and the fluorescence emitted at excitation wavelengths of 340 to 380 nm was detected by a charge-coupled device camera (Photonic Science, Millham, UK) at 6-s intervals and then stored. Fluorescence values from selected regions of the neuron that included the cell body were then analyzed offline in term of ratio value changes (R).

Drugs. β-Phenylethylamine, *p*-tyramine, phentolamine, reserpine, dopamine hydrochloride, AP-5, bicuculline methiodide, staurosporine, Rp-cAMPS, and SQ22,536 were purchased from Sigma (Milan, Italy); baclofen, CNQX, prazosin, sulpiride, SCH23390, carbidopa, U73122, and (*S*)-3,5- dihydroxyphenylglycine (DHPG) from Tocris Cookson Inc. (Bristol, UK); tetrodotoxin was from Alomone Labs (Jerusalem, Israel), CGP55845 was a kind gift from Novartis (Basel, Switzerland); phosphatidylinositol-3,4-biphosphate ($PtIns(4,5)P_2$) was purchased from Calbiochem (Sa Diego, CA); and Fura-2 was from Molecular Probes (Leiden, the Netherlands). All drugs were bath-applied at known concentrations by switching the perfusion using a three-way tap. Baclofen or DHPG (100 μM) was applied in extracellular solution through a glass pipette whose open end (approximately 2 μm in diameter) was positioned 20 to 30 μm from the cell body of the patched neuron. The local application of baclofen or DHPG was performed at 20 psi with a pneumatic picopump (Picospritzer; WPI). PtIns(4,5)P2 was dissolved in pipette solution at a nominal concentration of 500 μM. The solution was sonicated intermittently on ice for 30 min. Sonication was repeated each time before filling a new pipette. PtIns(4,5)P2 solutions were used for 1 day only. In addition, the PKA inhibitor c-AMPS-Rp/triethylammonium salt was applied by intracellular dialysis. A series of rats were treated with reserpine 5 to 8 mg/kg injected 15 to 24 h before the electrophysiological experiments. These dosages produced profound hypokinesias and ptosis.

Results

β-PEA and TYR Reduce the $GABA_B$ IPSP. Using intracellular recordings with sharp microelectrodes, we studied the effects of β-PEA and TYR on the slow inhibitory transmission mediated by $GABA_B$ receptors on dopaminergic cells ($n = 22$). Bath application of β-PEA and TYR reduced the amplitude of the $GABA_B$ IPSP (Fig. 1, a and b). This depressant action of β-PEA and TYR was observed in 19 (86%) of 22 cells, peaked in 4 to 6 min (Fig. 1d), was concentration-dependent (Fig. 1e), and was reproducible. The IC_{50} value for β-PEA and TYR were 43.8 ± 6.5 ($n = 10$) and 31.2 ± 3.3 μM ($n = 12$), respectively. The maximal inhibition caused by β-PEA (100 μM) was 41.5 ± 6.6% ($n = 10$), whereas that caused by TYR (100 μM) was 60.7 ± 4.5% ($n = 12$, $p < 0.05$). $GABA_B$ IPSP amplitude was also irreversibly reduced by the adrenergic antagonist phentolamine (30–100 μM) (Fig. 1c). Phentolamine has been shown recently to be a TA agonist (Bunzow et al., 2001). The inhibition caused by phentolamine

(100 μM) was 30 $\pm$ 6.7% ($n = 4$, $p < 0.05$). It is interesting that neither β-PEA nor TYR (100 μM) affected the amplitude of the evoked fast EPSP (Fig. 2a) or the $GABA_A$-mediated IPSP (Fig. 2b). In fact, the amplitude of the fast EPSP in control conditions (13.6 $\pm$ 2.3 mV) was unchanged in the presence of β-PEA (100 μM, 13.5 $\pm$ 2.7 mV, $n = 5$, $p = 0.29$) or TYR (100 μM, 12.2 $\pm$ 3 mV and $n = 5$, $p = 0.34$), and the amplitude of the $GABA_A$ IPSP (13.1 $\pm$ 1.2 mV) was not significantly modified by TYR (100 μM, 11.7 $\pm$ 1.4 mV, $n = 5$, $p = 0.19$) or β-PEA (100 μM, 12.3 $\pm$ 1.1 mV, $n = 5$, $p = 0.13$).

Responses to β-PEA and TYR after Catecholamine Depletion. Because TAs have been proposed to act as false transmitters displacing catecholamines from intracellular stores (Janssen et al., 1999; Mundorf et al., 1999), we tested whether a depletion of DA stores by pretreating the rats with reserpine (5–8 mg/kg i.p.) and perfusing the slices with carbidopa (300 μM) affects the reduction of the $GABA_B$ IPSP caused by β-PEA and TYR. Even under these conditions that abolish the indirect DA-mediated effects these TAs have on the dopaminergic cells (Geracitano et al., 2004), β-PEA and TYR still induced a clear-cut, reversible depression of the $GABA_B$ IPSP (Fig. 2c). In these conditions, the maximal inhibition caused by TYR (100 μM) was 32.1 $\pm$ 4.5% ($n = 7$), whereas that caused by β-PEA (100 μM) was 41.5 $\pm$ 6.6% ($n = 7$).

***β*-PEA and TYR Modulate $GABA_B$ Postsynaptic Responses.** We analyzed the effect of β-PEA and TYR on the $GABA_B$-mediated activation of the GIRK channel by using whole-cell patch-clamp recordings. Pressure applications of the $GABA_B$ agonist baclofen (100 μM, 1 s, every 2 min) in the vicinity of the soma of the recorded neuron (voltage-clamped at -60 mV) caused reproducible outward currents (135.3 $\pm$ 13.3 pA; $n = 23$) (Fig. 3a). Bath application of β-PEA (100 μM) and TYR (100 μM) reversibly reduced the baclofen-

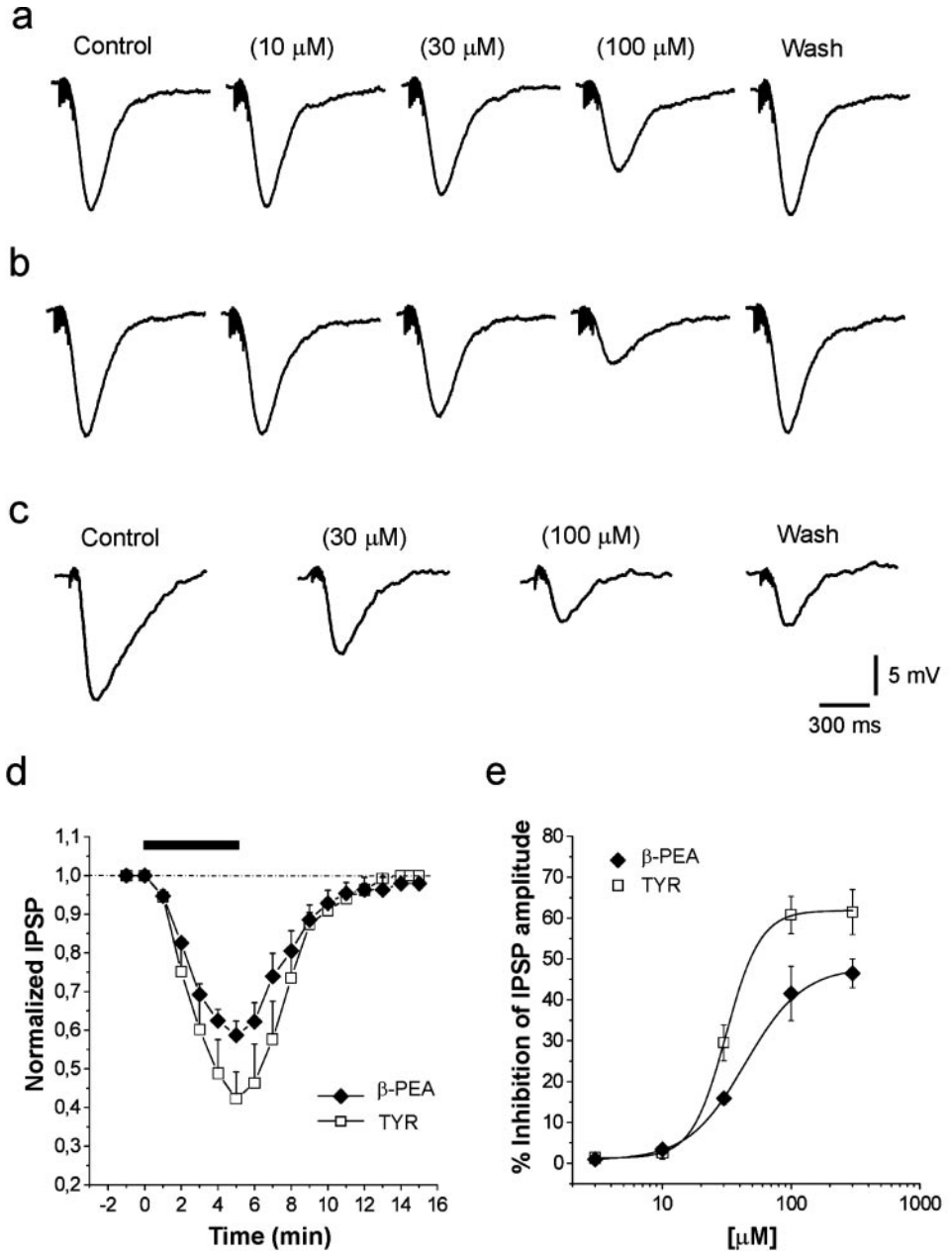

Fig. 1. TAs inhibit the $GABA_B$ IPSP. β-PEA (a) and TYR (b) reduce, in a concentration-dependent and reversible manner, the IPSP recorded with intracellular microelectrodes from DA neurons. c, phentolamine irreversibly reduces the IPSP (Each trace is an average of four sweeps). d, time course of the inhibition of $GABA_B$ IPSPs by β-PEA (100 μM, ♦) and TYR (100 μM, □) applied for the time indicated by the bar. Each data point represents the averaged IPSPs taken from 8 to 12 cells, each of which was normalized to the average amplitude of the IPSPs preceding TA superfusion. e, concentration-response curves of inhibition of the $GABA_B$ IPSP are shown for β-PEA and TYR. Each point represents the mean $\pm$ S.E.M. of $n = 12$ cells. The membrane potential during these and the subsequent intracellular experiments was maintained at -65 ± 2 mV by injecting hyperpolarizing current. The apparent input resistance was 107 $\pm$ 9 MΩ ($n = 12$).

activated outward current (IBac) by 55.6 ± 3.7% ($p < 0.001$; $n = 18$) and by 31.1 ± 3.9% ($p < 0.01$; $n = 10$), respectively, in 21 (91%) of 23 cells tested. β-PEA and TYR did not affect IBac responses in three cells. The time course of the effects of β-PEA and TYR on the IBac is shown in Fig. 3b. The maximal reduction of the amplitude of the IBac peaked after 4- to 6-min superfusion of β-PEA and 6- to 8-min superfusion of TYR. In addition, β-PEA and TYR caused a small inward current (35 ± 22 pA, $n = 18$) and did not modify membrane resistance. Bath application of DA (100 μM) (in the presence of DA antagonists) did not affect the baclofen response ($n = 3$, $p = 0.77$; data not shown). β-PEA and TYR also inhibited the outward current during continuous perfusion of baclofen (10 μM) to obtain a sustained activation of the GIRK channels. Thus, brief (3-min) coapplication of β-PEA (100 μM) or TYR (100 μM) together with baclofen (10 μM) induced a reversible reduction of the outward current of 30.5 ± 8 and 25 ± 6%, respectively ($n = 3$) (Fig. 3c). It is interesting that bath applications of the TA agonist phentolamine (100 μM) reduced IBac by 62.5 ± 3.4% within 8 to 10 min of perfusion. This effect did not reverse after 30 min of washout of the drug from the bath ($n = 7$, $p < 0.05$) (Fig. 3, a and b).

β-PEA and TYR Depress the Interaction of $GABA_B$ Gi/0 with GIRK Channels. To further analyze the mechanisms involved in the attenuation of GIRK by β-PEA and TYR, we loaded the dopaminergic neurons with the nonhydrolyzable GTP analog GTPγS trilithium salt (GTPγS, 0.3 mM) included in the intracellular solution of the patch pipette. The stimulation of the $GABA_B$ receptor leads to the substitution of GDP with GTP at the Gαi protein subunits, thus allowing the Gβγ subunits to dissociate and stimulate GIRK channels directly. The presence of GTPγS means that the Gαi subunits cannot reassociate with the Gβγ subunits to reverse their activation of GIRK (Kandel et al., 2000). Whole-cell perfusion with GTPγS activated a mean outward current of +205 ± 25 pA ($n = 6$) that gradually occluded nonreversing responses to transient $GABA_B$ receptor activation, indicating the saturated activation of GIRK by Gβγ subunits (Fig. 4, a and b). In these conditions, the mean holding current ($V_{hold} = -60$ mV) was + 221 ± 38 pA ($n = 13$). A subsequent 4- to 6-min bath application of β-PEA (100 μM) or TYR (100 μM) on cells either loaded with GTPγS or also treated with baclofen caused a sustained and nonreversible mean inward current of 202.5 ± 28 ($n = 7$) and 228.2 ± 31 pA ($n = 12$), respectively. These data indicate that β-PEA and TYR inhibited the GIRK channel, which had been tonically activated by the presence of GTPγS and/or $GABA_B$ receptor stimulation. It is interesting that the inward current induced by β-PEA and TYR did not reverse within the time course of recordings (up to 20 min after washout) in contrast to the reversible effects of β-PEA and TYR using normal GTP (Figs. 1–3). After the β-PEA–and TYR-induced inward current in GTPγS-treated cells, further application of baclofen was without effect (Fig. 4, a and b; $n = 11$). This suggests that β-PEA and TYR inhibit GIRK through a G-protein–dependent mechanism that cannot reverse because of the nonhydrolyzable nature of GTPγS. The inhibition of a tonically activated GIRK conductance by β-PEA and TYR was supported by analysis of the current-voltage relationship (I-V). In neurons loaded with GTPγS, current-voltage relationships were measured before and during bath application of the β-PEA and TYR. The net I-V for β-PEA (100 μM, $E_{rev} = -102 \pm 38$ mV, $n = 4$) and TYR (100 μM, $E_{rev} = -104 \pm 26$ mV, $n = 4$) indicate that trace amines specifically inhibit a potassium conductance (calculated reversal potential was −105 mV) (Fig. 4c). Consistent with the involvement of the GIRK channel, the β-PEA and TYR conductances showed inward rectification at hyperpolarized potentials (see the nonlinearity of the I-V plot).

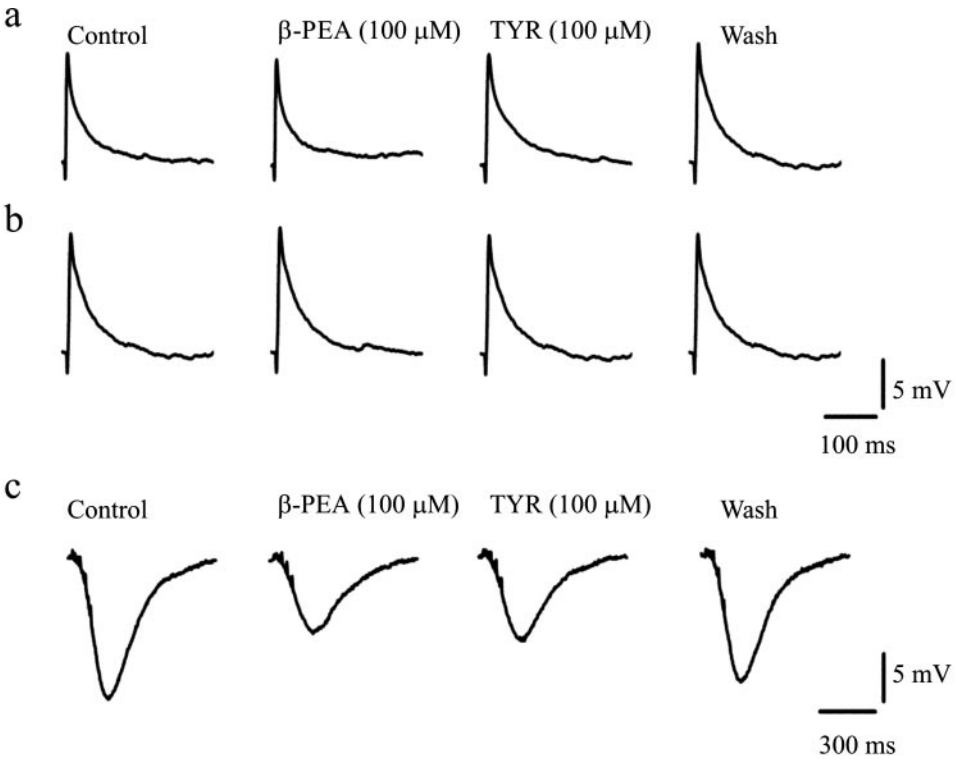

Fig. 2. TAs do not affect the ionotropic excitatory and inhibitory postsynaptic potentials but reduce the $GABA_B$ IPSP in DA-depleted slices. Neither the fast EPSPs (a) nor the $GABA_A$ IPSPs (b) are modified by β-PEA and TYR. $GABA_A$ IPSPs in b are depolarizing because of the hyperpolarized membrane potential. c, A DA-depleting treatment (reserpine 5–8 mg/kg i.p. and superfused carbidopa 300 μM for more than 30 min) does not affect the TA-induced inhibition of the $GABA_B$ IPSP.

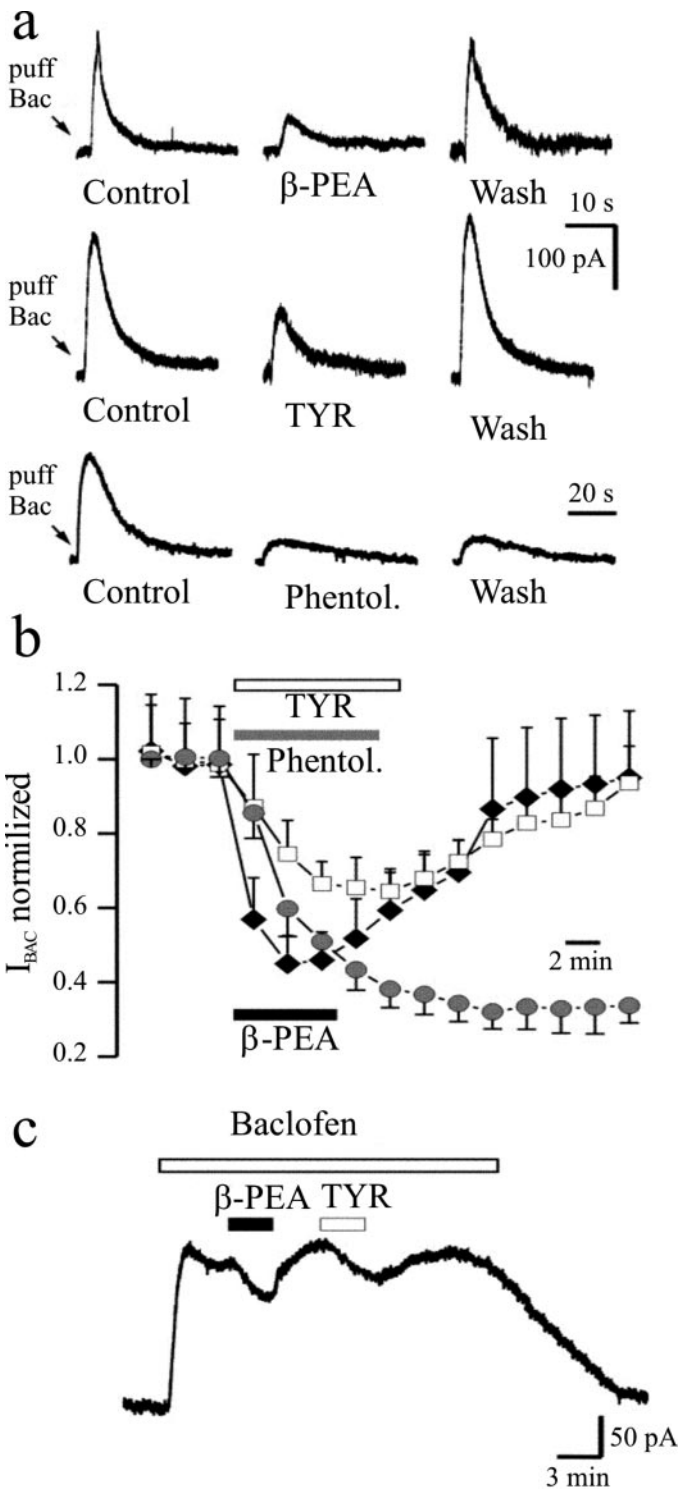

Fig. 3. TAs reduce the postsynaptic responses to $GABA_B$ receptor stimulation. a, outward currents caused by local pressure ejection applications of baclofen (puff Bac, arrows) are reversibly inhibited by β-PEA (100 μM, top traces) and TYR (100 μM, middle traces) and are not reversibly inhibited by phentolamine (Phentol., 100 μM, bottom trace). b, time course of the inhibition of the baclofen-elicited outward current by β-PEA (100 μM, ♦), TYR (100 μM, □), and phentolamine (100 μM, ○). Each point is obtained from 20 cells. c, reversible inhibition caused by a brief perfusion of both TAs on the outward current induced by a sustained bath application of baclofen on a dopaminergic neuron. The membrane potential during these and the subsequent patch-clamp experiments was maintained at −64 ± 2 mV.

PKA, PKC, PLC, and Intracellular Calcium Changes Do Not Mediate the Effects of β-PEA and TYR. Activation of the TA receptor has been associated with the enhancement of intracellular levels of c-AMP (Borowsky et al., 2001; Bunzow et al., 2001). To investigate the possible role of cAMP-PKA phosphorylation processes (Bonci and Williams, 1996; Couve et al., 2002) in the inhibition of baclofen responses by β-PEA and TYR, we tested blockers of the adenyl cyclase/cAMP/PKA pathway. Bath application of the adenyl cyclase inhibitor SQ22,536 (3 μM, 15–20 min) or the PKA-PKC inhibitor staurosporine (1 μM, 15–20 min) or the intracellular dialysis of neurons with the PKA inhibitor Rp-cAMPS, triethylammonium salt (Rp-cAMPS, 500 μM for 20 min) had no effect on the inhibition of the baclofen responses caused by either TYR (100 μM) or β-PEA (100 μM).

IBac was inhibited by β-PEA and TYR, respectively: 53.7 ± 4.1%, $p = 0.34$, and 24.5 ± 5.9%, $p = 0.54$, $n = 4$, in SQ22,536; 43.3 ± 3%, $p = 0.98$, and 33 ± 3.1%, $p = 0.79$, $n = 4$, in staurosporine; and 38.3 ± 3.8%, $p = 0.59$, and 25.8 ± 5.2%, $p = 0.53$, $n = 4$, with Rp-cAMPS.

We also considered the possibility that β-PEA and TYR inhibit GIRK channels via stimulation of PLC-mediated pathways. PLC hydrolyzes PtIns(4,5)P2 to yield the second messengers inositol(3,4,5)trisphosphate and diacylglycerol. The PLC inhibitor U73122 (20 μM, 12–16 min) superfused on the dopaminergic cells did not affect the reduction of IBac by β-PEA and TYR. In this condition, IBac was inhibited by β-PEA and TYR, respectively: 40.7 ± 3%, $p = 0.7$, and 32.7 ± 8.7%, $p = 0.82$, $n = 3$. The activity of U73122 was verified by its inhibition of 72 ± 10% ($n = 4$, $p < 0.01$; data not shown) of an outward current caused by puff applications of the selective mGluR-I agonist DHPG (100 μM, 50 ms). To investigate whether a PLC-induced depletion of PtIns(4,5)P2, which is believed to be required for activation of GIRK channels (Sui et al., 1998; Petit-Jacques et al., 1999), is involved in the TA-induced depression of the $GABA_B$-activated GIRK channel, we loaded the cells with PtIns(4,5)P2 via the patch pipette (Meyer et al., 2001). Inclusion of PtIns(4,5)P2 (nominally, 500 μM) had no effect on the amplitude or time course of IBac and did not significantly affect the β-PEA–and TYR-induced inhibition of the $GABA_B$-activated GIRK (35.7 ± 10.6%, $p = 0.63$, and 34.7 ± 8.7%, $p = 0.58$, $n = 3$).

Therefore, in line with the lack of effect of the PLC inhibitor U73122, there was no evidence that a depletion of PtIns(4,5)P2 is causally related to the β-PEA–and TYR-induced depression of the $GABA_B$-activated GIRK channel. Finally, a separate set of microfluorimetric experiments indicated that β-PEA and TYR did not modify the intracellular concentration of calcium. The mean ratio value for cytoplasmatic calcium remained unchanged in β-PEA (100 μM; Ca^{2+} ratio = 105.5 ± 9.6% of control, $n = 4$) or in TYR (100 μM; Ca^{2+} ratio = 109.8 ± 13.6% of control, $n = 4$; data not shown). All together, these results suggest that neither the activation of PKA, PKC, or PLC nor changes in cytoplasmatic calcium level represent the signaling step mediating the inhibitory effects of β-PEA and TYR on the $GABA_B$ receptor-activated responses.

Discussion

The results presented here demonstrate that β-PEA and TYR reversibly depress the slow $GABA_B$ IPSP in midbrain

dopaminergic cells, mainly by inhibiting the $GABA_B$ receptor-activated GIRK channels. Moreover, because this depression became irreversible in GTPγS-treated cells, we identify a G-protein–mediated role for β-PEA and TYR in modulating synaptic transmission in the mammalian central nervous system. Although the mobilization of DA from intracellular stores by β-PEA and TYR could be an important factor for the inhibition of the $GABA_B$ IPSPs (Federici et al., 2002), DA did not mediate the action of TAs because the β-PEA–and TYR-activated reduction in GIRK conductance was not mimicked by the superfusion of this catecholamine in the presence of classic DA antagonists. In fact, we demonstrate here that β-PEA and TYR still depressed the IPSP in reserpine- and carbidopa-treated cells in which the indirect (DA-mediated) effects of these TAs are abolished (Geracitano et al., 2004). Therefore β-PEA and TYR might activate specific TA receptors whose mRNA has been detected in the dopaminergic neurons of the ventral mesencephalon (Borowsky et al., 2001). The involvement of TA receptors is also supported by the observation that the unspecific and irreversible adrenergic antagonist phentolamine mimicked the effects of β-PEA and TYR on the dopaminergic cells, consistent with its reported agonistic action on TA receptors (Bunzow et al., 2001). Phentolamine is not expected to evoke DA-mediated effects like β-PEA and TYR or α1-mediated effects (prazosin was present) and is thus likely to be acting through TA receptor activation. In control conditions, TYR was more potent than β-PEA in reducing the $GABA_B$ IPSP, whereas a reverse pattern of potency was observed for the TA inhibition of GIRK currents. It is interesting that the effects of TYR and β-PEA on $GABA_B$ IPSPs after reserpine and carbidopa treatment showed a percentage inhibition almost identical with the inhibition of baclofen-activated postsynaptic currents. Thus, the difference in the TYR- and β-PEA–induced inhibition between synaptic and baclofen-evoked currents could be caused by additional effects of released DA (Federici et al., 2002).

It is generally assumed that GABA acting on $GABA_B$ re-

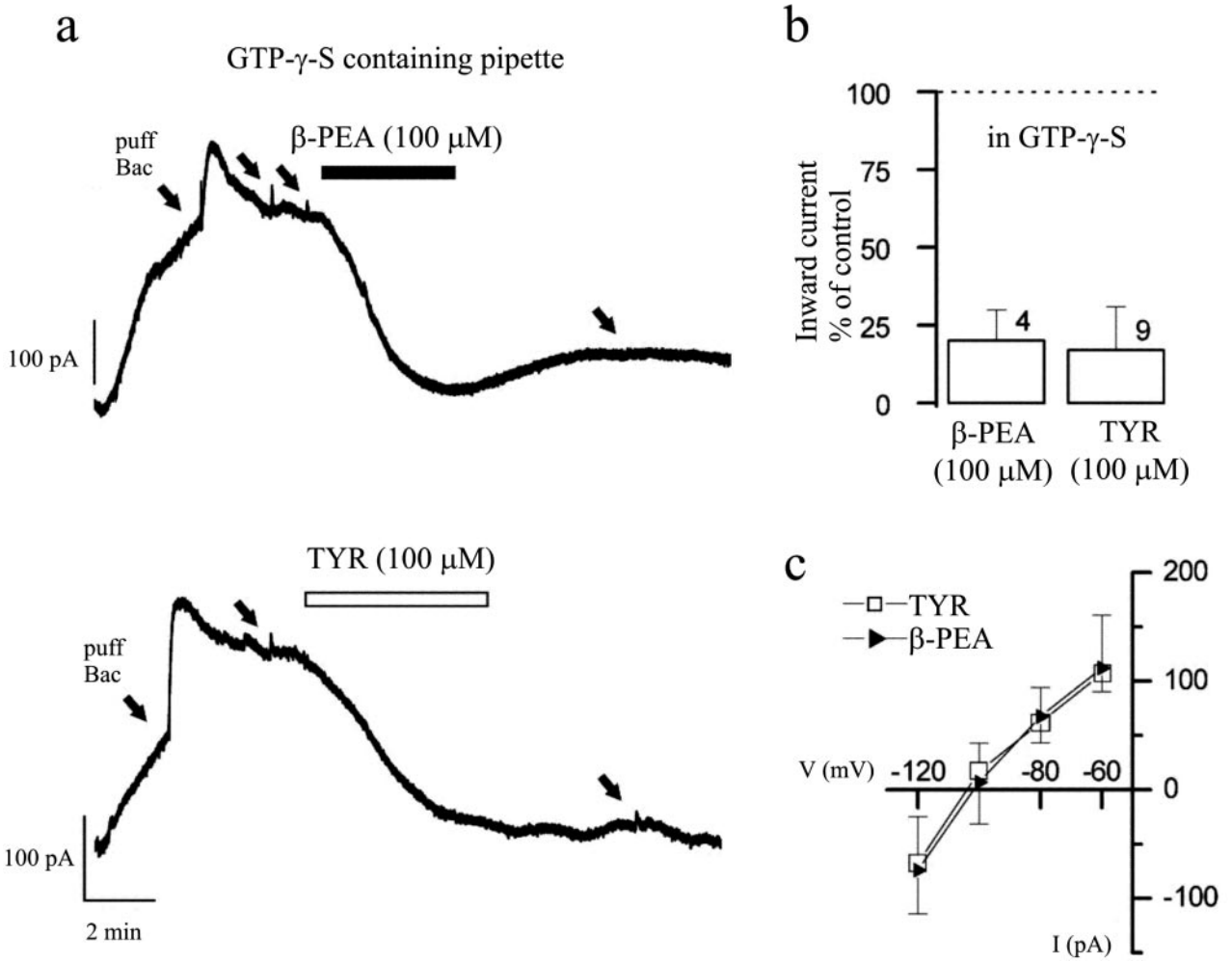

Fig. 4. A G-protein–linked mechanism mediates the inhibitory effects of TAs on the activated GIRK. The traces in a show currents recorded immediately after the establishment of whole-cell recordings. Intracellular solutions containing GTPγS (0.3 mM) activate a sustained outward current that occludes the outward responses to pressure ejection of baclofen (Puff Bac, arrows). Subsequent application of β-PEA (top trace) or TYR (bottom trace) inhibits the sustained outward current in an irreversible manner. b, the histograms summarize the inhibition by TAs of the outward current elicited by GTPγS and baclofen. Outward currents were measured relative to the holding current at the onset of whole-cell recordings and normalized with respect to the outward current present before TA application. Numbers next to bars indicate the number of experiments. c, net current-voltage relationships (I-V) for β-PEA and TYR represent the difference between I-V relationships measured at the peak of the outward current induced by GTPγS and after the application of the TA. These I-V relationships show a reverse potential consistent with E_K and inward rectification consistent with a reduction of GIRK.

ceptors dissociates the Gαi/0-βγ dimer, causing GIRK channel activation (Kunkel and Peralta, 1995; Huang et al., 1997; Lewohl et al., 1999). Therefore, in cells loaded with the nonhydrolyzable GTP analog GTPγS, the $GABA_B$ agonist baclofen induced a sustained opening of the GIRK channels that was irreversibly suppressed by TYR and β-PEA. This supports the notion that 1) the β-PEA and TYR inhibition of $GABA_B$ receptor-activated GIRK is a G-protein–dependent process, and 2) the persistent closure of the GIRK channels by TYR and β-PEA bypasses events taking place upstream of the dissociation of the βγ subunits activated by $GABA_B$ receptors. Thus, the nonreversible effects of TYR and β-PEA in the presence of GTPγS are not consistent with an action of these TAs directly at the $GABA_B$ receptor or a direct blockade of the GIRK channel, which are not likely to be affected by GTPγS.

It is interesting that the intracellular perfusion of GTPγS initiated a slowly developing outward current in dopaminergic neurons even in the absence of exogenous agonist application. This implies that tonic-activated GIRK-linked receptors, caused by spontaneous endogenous neurotransmitter release within the slice (e.g., $GABA_B$, nociceptin/orphanin FQ) (Lacey et al., 1988; Uchida et al., 2000), could be modulated by TYR and β-PEA.

The data presented here also demonstrate that PKA, PKC, PLC, or an increase in $[Ca^{2+}]_i$ is not causally involved in the inhibitory actions of TYR and β-PEA on GIRK channels. We therefore reasoned that a negative modulation of the activation the K^+ channels could account for the TYR- and β-PEA–induced inhibition of the baclofen-induced current. A similar negative regulatory mechanism has been suggested recently for the orexin-induced depression of neurotransmitter-activated GIRK channels in mammalian neurons (Hoang et al., 2003).

Therefore, the reduction of the $GABA_B$ IPSP might principally be caused by the activation of G-protein–coupled trace amine receptors that are stimulated by the trace amines TYR and β-PEA. Although the IC_{50} value for the reducing effects of trace amines is greater than the nanomolar range usually found in the brain (Berry, 2004), it could be possible that under particular metabolic conditions or pharmacological treatments (e.g., monoamine oxidase inhibition), the synaptic activity of $GABA_B$ receptors is regulated by TAs. Therefore, saturable, high-affinity binding sites for [*p*-^{3}H]tyramine (Ungar et al., 1977; Vaccari, 1986; Vaccari and Gessa, 1989) and β-[^{3}H]PEA (Nguyen and Juorio, 1989) have been reported in the nigrostriatal system.

Functional Implications. There is evidence suggesting that trace amines coexist with DA in the dopaminergic cells (Juorio et al., 1991) and activate motor activity by interacting with the dopaminergic system (Durden and Philips, 1980). The modulation of the GIRK channels by TAs might have a profound impact on integrative functions of the dopaminergic neurons to reduce the postsynaptic hyperpolarization caused by GABA released at inhibitory synapses. This suggests the involvement of trace amines in processes regulating motion, emotion, and reward (Borison et al., 1977; Sabelli et al., 1978; Wise and Bozarth, 1987; White, 1996). In particular, the shaping of GABA inputs could be an important phenomenon for the development of addiction, because it has been demonstrated recently that drugs of abuse alter $GABA_B$ receptor transmission in the ventral midbrain (Giorgetti et al., 2002) and activate TA receptors (Borowsky et al., 2001).

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