Spontaneous Apamin-Sensitive Hyperpolarizations in Dopaminergic Neurons of Neonatal Rats

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INTRODUCTION

A large body of literature shows that electrophysiological properties of mammalian neurons undergo developmental changes. For example, modifications in the kinetics of synaptic currents (Carmignoto and Vicini 1992) and in fundamentally regulated physiological properties (i.e., inhibitory or excitatory) of neurotransmitter systems (Leinekugel et al. 1997) were demonstrated. We have undertaken an in vitro study to address this issue in midbrain dopaminergic (DA) neurons. Here, we report that DA neurons of young rats exhibit spontaneous hyperpolarizations that are mostly absent in adults, and we characterize their pharmacology.

METHODS

Methods were similar to the ones used in a previous paper (Seutin et al. 1997) with a few modifications. Both neonatal (9–16 days, n = 25) and adult (2-mo-old, n = 8) Wistar rats were used. They were housed and handled in accordance with the Guide for the Care and Use of Laboratory Animals, Publication No (NIH) 85–23, Revised 1985. To exclude the possibility that the phenomenon observed in our study was due to the anesthetic, some (n = 16) neonatal rats were killed without prior anesthesia. The other animals were anesthetized with halothane. All animals were decapitated.

Horizontal slices were prepared as described (Seutin et al. 1997), and the same landmarks were used to localize the substantia nigra. Slices were completely submerged in a solution of the following composition (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 11 glucose, and 18 NaHCO3, saturated with 95% O2–5% CO2 (pH 7.4). Flow rate and temperature were set at 2.5 ml/min and 35 ± 0.5°C, respectively. All drugs were bath applied.

Intracellular recordings were made with microelectrodes (resistance: 60–150 MΩ) filled with 2 M KCl. In some cases, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′′-tetraacetic acid. They were resistant to tetrodotoxin in the majority of the cells. They bridge balance mode at approximately Seutin et al. 1997) with a few modifications. Both neonatal (9–16 rats (19 neurons without prior anesthesia and 11 neurons days, 80: 3361 ± 3364, 1998. Intracellular recordings from substantia nigra slices revealed the existence of spontaneous hyperpolarizations (amplitude 2–8 mV, duration 100–400 ms) at −60 mV in most dopaminergic neurons of neonatal (9–15 days) but not adult rats. These events were blocked by apamin (300 nM) and bicuculline methochloride (100–300 μM), which blocks apamin-sensitive currents. They were unaffected by the selective γ-aminobutyric acid-A (GABAa) antagonists SR95531 (100 μM) and picrotoxin (30–50 μM), the GABAa antagonist CGP35348 (300 μM), the D2 antagonist haloperidol (1 μM), and the metabotropic antagonist MCPG (1 mM). The hyperpolarizations were strongly attenuated or abolished when recording electrodes contained 200 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′′-tetraacetic acid. They were resistant to tetrodotoxin in the majority of the cells. They had some voltage dependency and were in some cases transiently potentiated when cells were briefly depolarized by current injection. We conclude that dopaminergic neurons have developmentally regulated physiological properties. These spontaneous hyperpolarizations might affect the firing rate of these cells, which was found to be lower in neonates than in adults.

RESULTS

Experiments were obtained from 30 neurons of neonatal rats (19 neurons without prior anesthesia and 11 neurons with prior anesthesia) and 8 neurons from adult rats. All recorded cells met electrophysiological and pharmacological criteria for DA neurons, including a spontaneous, pacemaker-like firing (except for 3 cells from neonates that were quiescent), a broad spike followed by a long-lasting apamin-sensitive AHP, a large Is current (which was identified by a prominent sag in voltage during a long hyperpolarizing current injection), and a hyperpolarizing effect of the D2 antagonist haloperidol (1 μM). The GABAa antagonist CGP35348 (300 μM), the D2 antagonist haloperidol (1 μM), and the metabotropic antagonist MCPG (1 mM) were used as blockers. We have recently shown that these concentrations of the drugs fully block the AHP (Seutin et al. 1997).

Drugs used and their supplier were as follows: apamin, BMC, picrotoxin, cesium chloride, BAPTA (Sigma); tetrodotoxin (TTX) (ICN Biomedicals, Aurora, OH); MCPG (α-methyl-4-carboxyphenylglycine) (Research Biochemicals International), SR95531 (2-[carboxy-3'-propyl]-3-amino-6-paramethoxy-phenyl-pyridazinium bromide) (gift from Sanofi-Winthrop), BHT920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]-azepin) (gift from Boehringer Ingelheim), haloperidol (gift from Janssen Pharmaceutica), and CGP35348 [(P-(3-aminopropyl)-P-diethoxy-methyl-phosphinic acid; gift from Ciba-Geigy).

All data are expressed as means ± SE. Statistical differences were assessed with the χ2 test or Student’s t-test. Differences were considered significant at P < 0.05.
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FIG. 1. Apamin and bicuculline methochloride (BMC) block the spontaneous hyperpolarizations. Chart recording of 2 dopaminergic neurons in slices taken from neonatal rats. The membrane potential was brought to approximately −60 mV by negative current injection (−180 pA on the left and −150 pA on the right). The effect of BMC was quickly reversible (wash taken after 7 min).

agonist BHT920 (Johnson and North 1992; Mercuri et al. 1995; Yung et al. 1991). Interestingly, the firing rate of DA neurons was lower in neonates (1.5 ± 0.2 spikes/s in the whole group and 1.4 ± 0.2 spikes/s in the group where halothane anesthesia was used) than in the adults (2.6 ± 0.3 spikes/s, P < 0.01 for both comparisons). The input resistance was similar in both groups (258 ± 23 MΩ in the neonates and 241 ± 22 MΩ in the adults; NS).

When DA cells of neonatal rats were hyperpolarized to −60 mV by DC current injection, spontaneous hyperpolarizations were observed in 26 of 30 neurons (16/19 in slices from nonanesthetized animals and 10/11 in slices from anesthetized animals, P > 0.05 between the 2 groups; Figs. 1–3). These events occurred irregularly, with a frequency in the range of 0.3–1.5 Hz and had an amplitude and a duration of 2–8 mV and 100–400 ms, respectively. They were rarely preceded by a short depolarizing event. On the contrary, such hyperpolarizing events occurred in only one of eight cells from adult animals (P < 0.005 vs. the neonates). This was apparently not due to differences in the density of apamin-sensitive channels; indeed, the mean area under the curve for the AHP in neonates (n = 4) was 1.05 times that observed in adults (n = 8) (data taken from Seutin et al. 1997) (NS). On the other hand, these events were easily distinguished from much slower oscillations that occur both in neonates and in adults when the membrane potential is brought closer to the action potential threshold (−40 to −45 mV) (not shown).

The hyperpolarizations were blocked by apamin (300 nM, n = 5), a specific blocker of SK-type Ca2+-activated K+ channels and BMC (100–300 μM, n = 6), which also blocks apamin-sensitive currents (Johnson and Seutin 1997). As shown in Fig. 1, the effect of BMC, but not that of apamin, was quickly reversible, as already described (Seutin et al. 1997).

On the other hand, the hyperpolarizations were unaffected by the γ-aminobutyric acid-A (GABA_A) antagonists SR95531 (100 μM, n = 3) and picrotoxin (30–50 μM, n = 3). These concentrations of both drugs are above those needed to completely block GABA_A receptors in these cells (Seutin et al. 1997). The GABA_A antagonist CGP35348 (300 μM, n = 3) and the D2 antagonist haloperidol (1 μM, n = 3) also did not inhibit the spontaneous hyperpolarizations, whereas they did completely antagonize the hyperpolarization induced by the GABA_B agonist baclofen (n = 5) and the D2 agonist BHT920 (n = 5), respectively (Fig. 2). Because of the recent report of a synaptic potential caused by the activation of metabotropic receptors and mediated by apamin-sensitive channels (Fiorillo and Williams 1998), we also tested the effect of the broad antagonist MCPG (1 mM, n = 3), which was ineffective.

In the majority of cells, the hyperpolarizations were resis-

FIG. 2. Lack of blockade of the spontaneous hyperpolarizations by a γ-aminobutyric acid-B and a D2 antagonist. Top traces: CGP 35348 blocks the effect of baclofen, and haloperidol blocks the effect of BHT920. Starting membrane potential was −60 mV (DC current injection: −110 pA). Control experiments revealed that the hyperpolarization induced by the agonists was stable over time in the absence of antagonists (not shown). Bottom traces: both agents fail to block the spontaneous hyperpolarizations (DC current injection: −110 pA).
FIG. 3. Tetrodotoxin does not block the hyperpolarizations. Experiments on 2 different neurons are shown. Amplitudes of current injection were -120 pA (top traces) and -130 pA (bottom traces).

Interestingly, the firing rate was slower in neonatal than in adult DA neurons, an effect that could be expected from spontaneous hyperpolarizations occurring at approximately -60 mV. However, demonstrating a causal relationship between these two observations would require a blocker of the hyperpolarizations that would not affect the AHP as well as an extensive comparison of the currents that influence the spiking activity in these cells. A lower firing rate of DA neurons in neonates has also been shown in a previous in vivo study (Tepper et al. 1990).

The exact nature of the hyperpolarizations remains to be established. TTX did not block their occurrence, except in two cells in which their shape was atypical. This suggests that there may be some heterogeneity in the origin of these hyperpolarizations. However, for the majority of the events, a synaptically induced potential or a potential mediated by electrical coupling between cells seem to be unlikely. Moreover, the hyperpolarizations were strongly attenuated or abolished when the recording electrode contained the Ca2+ chelator BAPTA. We therefore favor the possibility that the K+ channel openings are due to a rise in intracellular Ca2+ after its release from stores or its entry through channels. Experiments are in progress to test these hypotheses.

Finally, it is intriguing that previous in vitro investigations on DA neurons of young animals did not detect the events that we describe. However, these studies used whole cell patch-clamp recordings, and, in preliminary experiments, we also failed to detect them reliably by using this technique.

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