Brain Research, 367 (1986) 230–237
Elsevier

BRE 11508

Autoreceptor-Mediated Changes in Dopaminergic Terminal Excitability: Effects of Potassium Channel Blockers

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(Accepted July 9th, 1985)

Key words: dopamine — autoreceptor — transmitter release — 4-aminopyridine — tetraethylammonium

The effects of the potassium channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA), on autoreceptor-mediated changes in dopaminergic terminal excitability were examined in urethane-anesthetized rats. Local infusions of 4-AP or TEA into neostriatal terminal fields of nigral dopaminergic neurons led to marked decreases in terminal excitability, as measured by the increase in stimulating current required to activate the neurons antidromically from the site of the infusion. The decreased excitability resulting from 4-AP could be reversed by subsequent i.v. injection of haloperidol, and was blocked in rats that had been depleted of endogenous dopamine by prior treatment with alpha-methyl-*p*-tyrosine (AMpT). Thus, the decrease in excitability elicited by the potassium channel-blockers was indirect, and apparently due to increased autoreceptor stimulation resulting from enhanced transmitter release. In addition, co-infusion of 4-AP and apomorphine in AMpT-treated animals led to decreased terminal excitability that did not differ from the effects of apomorphine alone, indicating that 4-AP did not block the effects of exogenous autoreceptor agonist administration. These results provide in situ electrophysiological evidence that autoreceptor-mediated processes occurring at dopaminergic terminals are not mediated by 4-AP- or TEA-sensitive potassium channels. Furthermore, our findings suggest that, as in other types of presynaptic terminals, blockade of voltage-sensitive potassium channels in dopamine terminals leads to enhanced release of transmitter.

INTRODUCTION

The amount of transmitter released from monoamine terminals following presynaptic depolarization has been hypothesized to be subject to feedback inhibition via the stimulation of presynaptic autoreceptors, located on or near to the sites of transmitter release[4,6,8,13,26]. Although the precise mechanism by which autoreceptor stimulation leads to a decrease in transmitter release is not well understood, recent data have indicated that the inhibition of release is associated with a decrease in the excitability of monoamine terminals to electrical stimulation in vivo[11,19,20,22,23,28–31], suggestive of a presynaptic hyperpolarization and/or increase in membrane conductance[15,34]. Consistent with these observations, intracellular recordings from monoamine cell bodies, which possess autoreceptors that are pharmacologically similar or identical to those at the terminals, reveal that autoreceptor stimulation leads to somadendritic hyperpolarization[1,2,9,36].

Although the ion(s) responsible for autoreceptor-mediated hyperpolarization in dopaminergic cell bodies has not yet been identified, in noradrenergic and serotonergic neurons, autoreceptor-mediated hyperpolarizations are due to a specific increase in conductance to potassium[1,2,36]. The nature of this potassium conductance change, and its susceptibility to blockade by pharmacological agents, are unknown, but a hyperpolarization mediated by an increase in potassium conductance at the nerve terminal could account for decreased terminal excitability and decreased transmitter release following terminal autoreceptor stimulation in monoamine neurons, similar to its effects in *Aplysia*[24]. Several distinct classes of voltage-sensitive potassium channels have been dem-

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onstrated in various neurons, which can be blocked by the external application of the drugs 4-AP or TEA[3,32,33], and in *Aplysia*, 4-AP blocks the presynaptic modulation of transmitter release[24].

The present experiments were carried out in an attempt to elucidate the ionic mechanisms underlying dopamine autoreceptor-mediated decreases in terminal excitability. Striatal dopamine terminals are subject to endogenous autoinhibition in situ from dopamine released as a consequence of ongoing neuronal activity, as indicated by increases in terminal excitability subsequent to administration of dopamine autoreceptor antagonists or coincident with decreases in neuronal firing rate[29,30]. If terminal autoreceptors regulate the level of polarization and excitability of dopamine terminals through 4-AP- or TEA-sensitive potassium channels, then application of these potassium channel blockers would be expected to block endogenous terminal autoinhibition, thereby leading to increased dopaminergic terminal excitability, similar to the effects of dopamine antagonists[11,29,30].

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats, housed 2 or 3 to a cage, weighing between 230 and 350 g at the time of recording, were the subjects for all experiments. Animals were anesthetized with 1.3 g/kg urethane (ethyl carbamate), i.p., and in some cases, the left femoral vein was cannulated for i.v. drug administration. Further details on animal preparation were as described previously[29].

Electrical stimulation and drug infusions

Stimulating electrodes consisted of bipolar stainless-steel enamel-coated wires, approximately 200 μm in diameter, with a tip separation of approximately 100 μm. Electrical stimuli to the ipsilateral dorsal anterior neostriatum (0.5 to −0.5 mm anterior to bregma, 3.5 mm lateral to the midline, 3.5 to 4.0 mm ventral to the cortical surface) consisted of single monophasic square-wave pulses of durations ranging from 10 to 500 μs at 0.3–3.0 mA, delivered at a rate of 1/s. Stimulating electrodes were individually calibrated in situ prior to each experiment, and were monitored periodically throughout all experiments to control for possible changes in impedance. A 32-gauge infusion cannula was connected by a short length of Teflon tubing to a 10 μl Hamilton microsyringe, controlled by a Harvard Apparatus infusion pump. The infusion cannula and the stimulating electrode were held in place with a small Narishige micromanipulator, and aligned so that the open end of the cannula was within 200 μm of the exposed tips of the stimulating electrode. Infusions consisted of a volume of 0.31 μl, delivered over the course of 5 min. In some cases, drugs were infused into, and excitability tested from preterminal regions of nigrostriatal axons in the medial forebrain bundle (MFB, 4.2 mm anterior to lambda, 1.75 mm lateral to the midline, 7.8 mm ventral to the cortical surface).

Drugs

The drugs used in these experiments were as follows: 4-aminopyridine (Sigma), 0.1 or 1.0 mM; tetraethylammonium chloride (Sigma), 100 mM; apomorphine (Sigma), 10 μM; and, for i.v. administration, haloperidol lactate (McNeil), 0.05–0.10 mg/kg. All drugs were dissolved in 0.9% saline, except for apomorphine which was prepared in 0.9% saline in 0.1% ascorbate. In some cases, rats were pretreated with alpha-methyl-*p*-tyrosine (AMpT; Sigma, 250 mg/kg, i.p.) 18 and 3–6 h prior to excitability testing in order to deplete endogenous levels of dopamine[17].

Recordings

Extracellular single unit recordings of electrophysiologically identified dopaminergic neurons[5,9,12,29,37] in substantia nigra pars compacta (2.1 mm anterior to lambda, 2.0 mm lateral to the midline, 6.8 to 7.5 mm from the dorsal surface of the brain) were obtained with glass micropipettes, filled with 3 M NaCl and possessing in vitro impedances of 4–10 MΩ at 500 Hz. Upon encountering dopaminergic neurons, the stimulus current and/or duration were varied in order to determine if the cell could be driven antidromically. Responses were considered to be antidromic provided that they collided with appropriately timed spontaneous discharges[7]. In addition, antidromic responses of dopaminergic neurons were characterized by their slow conduction velocity, constant latencies, and usually consisted of the initial segment spike only, as described previously[5,9,12,29]. Data were recorded on magnetic tape for off-line analysis.